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16. ABSTRACT

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SENSITIVE BIOCHEMICAL AND BEHAVIORAL INDICATORS OF TRACT SUBSTANCE EXPOSURE - PART II, PLATINUM

by

Edward J. Massaro
Pennsylvania State University
Center for Air Environment Studies
University Park, Pennsylvania 16802

Bradley A. Lown, John B. Morganti
Department of Psychology
State University College at Buffalo
Buffalo, New York 14222

Carl H. Stineman, Rosemary B. D'Agostino
Department of Biochemistry
School of Medicine
State University of New York at Buffalo
Buffalo, New York 14214

68-02-1768

Project Officer

George M. Goldstein
Human Studies Division
Health Effects Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, North Carolina 27711

Health Effects Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, North Carolina 27711

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FOREWARD

The many benefits of modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory participates in the development and revision of air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

With the advent of the automotive catalytic convertor as a means of reducing mobile source emissions, research was undertaken to define the pharmacological and behavioral effects of the convertor attrition products, platinum and cerium. This data, in conjunction with our health studies, will allow EPA to assess the potential health risks of these substances.

F. Gordon Hueter
Director
Health Effects Research Laboratory

ABSTRACT

The tissue/organ distribution of platinum (Pt) was examined in the adult mouse at various times after exposure to single or serially repeated (multiple) doses of $Pt(SO_4)_2$ or Na_2PtCl_6. Effects of exposure to Pt on selected parameters of open field, exploratory and social behavior and passive and active avoidance learning were investigated. Effects of Pt on the gravid female (per se) and on the embryo/fetal and postnatal development of offspring of Pt exposed dams also were investigated.

Via the intragastric (i.g.) route of administration, disproportionately high tissue Pt levels were found in animals receiving $Pt(SO_4)_2$ at the LD_{25} level compared to the LD_5 level. Damage to the GI tract at the higher dose level may facilitate Pt absorption resulting in exceptionally high blood Pt levels. The high blood levels may damage the kidneys, decreasing Pt excretion, resulting in increased tissue uptake.

Analysis of performance in the open field indicated that expousre to a single i.g. LD_{25} depressed ambulations significantly and rearings marginally from 4 hours through 7 days post administration. No effect on exploratory behavior was observed. Highly significant negative correlations were found between behavioral performance and tissue Pt levels for all tissues except brain and muscle suggesting that Pt may exert a disruptive influence on cellular processes which is expressed as general malaise and depressed behavior.

Platinum sulfate had no effect on social behavior, wheel running performance, passive avoidance learning or acquisition of two-way active avoidance learning.

The tissue/organ Pt levels of adult male mice exposed to multiple doses (one LD_5 every 72 hours for up to 10 doses) of $Pt(SO_4)_2$ were highly variable, but generally increased with dose number. No Pt was detected in the brain. General activity and exploratory behavior were marginally depressed and only rearings were significantly correlated with tissue Pt levels. This correlation did not involve the brain, indicating a general systemic effect, rather than a neurotoxic effect.

In utero exposure to $PtSO_4$ (day 7 or 12 of gestation) resulted in reduced pup weight which carried over to adulthood (day 45 post partum). Lactational exposure resulted in reduced neonatal activity and reduced adult weights.

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Exposure of the adult mouse to sodium hexachloroplatinate (Na_2PtCl_6) at the LD_5 and LD_{25} levels depressed ambulations and rearings in the open field at 4 hours post administration. No effects on exploratory behavior were observed. Gestational exposure (Day 12 only) resulted in reduced neonatal activity and complex effects on adult behavior.

This report was submitted in fulfillment of Contract No. 68-02-1768 by the State University of New York at Buffalo under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period April 18, 1975 to April 3, 1979.

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INTRODUCTION

PLATINUM

Until relatively recently, human exposure to platinum (Pt) and Pt compounds has been limited mainly to the workplace (e.g. mining, refining and the manufacture and reprocessing of catalysts). However, beginning with the introduction of the automobile catalytic converter (1975 automobile model year), the therapeutic use of Pt complexes with anti-tumor activity (1) and the inevitable increase in coal utilization [which, may release significant quantities of Pt(2)], a potential for widespread, long-term environmental dissemination and increased bioavailability of Pt exists. A study by the U.S.E.P.A. of the exhaust from vehicles equipped with catalytic converters found no Pt in the airborne particulates collected on filters (detection limit $\sim 10^{-5}$ µg/g) and bench testing of engines by the New York State Department of Environmental Conservation found Pt emissions to be in the micrograms per mile range (3). Similarly, a study conducted by General Motors found airborne Pt emissions to range from 1-3 µg/mile (2). However, larger particulates are emitted from catalytic exhaust systems (3). This material is deposited on road surfaces and adjacent land areas, is a component of fugitive dust and ultimately enters surface waters. Widely varying Pt concentrations have been found in this material: up to 635 µg Pt/g (3). Chemical analysis has demonstrated that some of this Pt is in the +4 oxidation state; but no quantification or chemical characterization was reported (3). In addition, Wood has suggested that Pt could be solubilized via methylation by specific microorganisms (in a manner similar to mercury) greatly enhancing its bioavailability (4).

It is of considerable interest that numerous environmentally available toxicants may have the ability to modify behavior in an insidious manner, without producing overt, symptoms of intoxication. In this context, nothing is known about Pt compounds. Thus, we undertook to study the potential behavioral effects of exposure to Pt salts employing the mouse as a model mammalian system.

Scope of the Research

The overall objective of this project was to characterize Pt [$Pt(SO_4)_2$, Na_2PtCl_6] intoxication in a model mammalian system (the mouse) employing acute (single) and serially repeated (multiple) dose exposure paradigms. Platinum lethality parameters were defined in both sexes of the adult mouse and Pt tissue/organ distribution was monitored as a function of time and dose. In coordination with the tissue distribution study, investigations of the effects of Pt on selected parameters of open field, exploratory and social

behavior, passive avoidance learning and the acquisition of two-way active avoidance learning were undertaken. Correlations among tissue levels of Pt and open field behavioral parameters (ambulations and rearings) were probed employing statistical methodology.

Since numerous xenobiotics are known to cross the placenta and/or accumulate in maternal milk, studies of Pt tissue/organ distribution in the gravid female and the embryo/fetus/offspring were undertaken. Platinum levels were monitored as a function of time. Coordinate studies examined developmental and behavioral parameters of the offspring (as neonates, pups and adults) of exposed mothers. In addition, maternal behavior was examined as a function of pup retrieval and neonate and pup activity levels were assessed. Ambulations and rearings in the open field, passive avoidance learning and rotarod performance were assessed in adult offspring of exposed mothers.

I. LETHALITY STUDIES

Lethality parameters for Na_2PtCl_6 and $Pt(SO_4)_2$, administered intragastrically (i.g.), subcutaneously (s.c.) and intravenously (i.v.), were established in the adult male and female mouse. The i.g. route of administration was selected because of its environmental relevance and the s.c. and i.v. routes because they should provide better dose control. In addition, the s.c. route is related to the transdermal route and, therefore, dosage has environmental relevance.

A. Na_2PtCl_6 AND $Pt(SO_4)_2$ ADMINISTERED TO ADULT MALE MICE

1. Na_2PtCl_6

MATERIALS AND METHODS

Animals

Six to 8 week old (27-33 gm) random bred male Swiss-Webster ICR mice (West Seneca Laboratories, West Seneca, NY) served as subjects. The animals were housed 5/cage in suspended wire cages and acclimated to the laboratory environment for at least 1 week prior to experimental manipulation. Food (Agway 1000 Rat and Mouse Maintenance Diet) and tap water were freely available.

Chemicals

All chemicals were of the highest grade available commercially. When indicated, analysis and purification were performed employing established procedures.

Dosing

A series of solutions of logarithmically increasing Na_2PtCl_6 concentrations was prepared in deionized glass distilled water for each route of administration (i.g., i.v., s.c.). pH was adjusted to 7.4 with NaOH. The solutions were sterilized by autoclaving at 121°C and 20 psi for 30 minutes and were administered to groups of 10 randomly selected animals in a volume equivalent to 1% (v/w) of body weight. Control groups received HCl solutions of identical pH. The pH of these solutions was found to be unstable; decreasing by approximately 1 pH unit in 48 hours with a concommitant increase in toxicity. Therefore, an investigation was undertaken of the toxicity of unneutralized Na_2PtCl_6 solutions, the pH of which ranged from

2.40 to 2.52 depending on Na_2PtCl_6 concentration. The low pH of these solutions precluded investigation of the i.v. and s.c. routes of administration. Therefore, only the i.g. route was investigated.

Protocol

The number of deaths in each group were recorded through 7 days post administration. Lethal dose percentiles (LD_{xx}) and confidence intervals were obtained by the method of probit analysis [as described by Finney (5) using the Isobol computer program of Gessner and Cabana (6)] for each route of administration.

RESULTS

The LD_{50} levels obtained by probit analysis for neutralized solutions were: i.g. = 75.9 [64.2 - 303.6, 90% (the 95% confidence interval could not be obtained in this case because the percentage of deaths did not increase monotonically with dose)] mg Pt/kg; i.v. = 21.0 (19.4 - 22.7, 95%) mg Pt/kg and s.c. = 26.4 (24.3 - 28.7, 95%) mg Pt/kg. The LD_{50} for unneutralized Na_2PtCl_6 administered i.g. was 82.5 (72.1 - 108.5, 95%) mg Pt/kg. No deaths occurred in the HCl control groups.

2. $Pt(SO_4)_2$

Animals

The mice were selected and handled as described above in the Na_2PtCl_6 study.

Chemicals

All chemicals were of the highest grade available commercially. When indicated, analysis and purification were performed employing established procedures.

Dosing

A series of logarithmically increasing $Pt(SO_4)_2$ concentrations were prepared in deionized glass distilled water. Since $Pt(SO_4)_2$ is insoluble at neutral pH, the solutions could not be neutralized. The low pH (0-2) of these solutions precluded investigation of the i.v. and s.c. routes of administration.* Therefore, only the i.g. route was investigated. The solutions were autoclaved and administered to groups of 10 randomly selected animals in a volume equivalent to 1% of body weight. Control groups received H_2SO_4 solutions at the same pH (adjusted with NaOH) and sulfate concentrations as the Pt experimental groups.

* Severe necrotic lesions developed at s.c. injection sites and death rapidly followed i.v. administration of doses as low as 10 ppm. Subcutaneous administration is inappropriate for investigating behavioral effects of $Pt(SO_4)_2$ because the lesions produced at the site of injection would, most likely, confound any behavioral alterations that might be induced.

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Protocol

Lethal dose percentiles were obtained as described above for the Na_2PtCl_6 study.

RESULTS

The 7 day i.g. LD_{50} for $Pt(SO_4)_2$ [pH range = 1.06 (150 ppm Pt) to 0.46 (500 ppm Pt)] was 280.5 (237.6 - 320.1, 95%) mg Pt/kg. No deaths occurred in control groups.

B. Na_2PtCl_6 ADMINISTERED TO GRAVID AND NONGRAVID FEMALE MICE

MATERIALS AND METHODS

Animals

Six to 8 week old (27-33 gm) female Swiss-Webster ICR mice were maintained as described above for male mice. Following at least 1 week of acclimation randomly selected subjects were bred to competent males. Observation of a vaginal plug defined day 0 of pregnancy. Pregnant females were housed individually on wood chip bedding and allowed access to food and water *ad libitum*. Dams that were to be used in *post partum* experiments were allowed to deliver and their litters were immediately culled to 6 pups; 3 males and 3 females. Delivery defined day 1 *post partum*.

Chemicals

As described above in the Na_2PtCl_6/adult male study.

Dosing

Neutralized solutions (pH 7.4) were prepared (as described above in the Na_2PtCl_6/adult male study) immediately before use. Experimental animals received from 15 to 45 mg Pt/kg s.c. in a volume equivalent to 0.2 ml/30 gm of body weight. Controls received phosphate buffered saline (PBS: 0.13 M NaCl, 0.01 M Na_2HPO_4/NaH_2PO_4, pH 7.4).

Protocol

Randomly selected nongravid and gravid (day 12 of gestation) females were assigned to test and control groups comprised of 10 animals. Viability through 7 days post administration was tabulated (Table 1) and lethal dose levels (Table 2) were calculated by linear regression analysis.

RESULTS

Analysis of the data (Table 2) shows that the gravid and nongravid animals were equally susceptible to Pt toxicity. LD_{50} values of 32.4 and 30.0 ppm Pt were obtained for gravid and nongravid animals, respectively.

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II. TISSUE DISTRIBUTION STUDIES

A. $Pt(SO_4)_2$-SINGLE (ACUTE) DOSE STUDY

MATERIALS AND METHODS

Animals

Sixteen groups of ten 6-8 week old male mice were investigated. They were maintained and handled as previously described.

Dosing

$Pt(SO_4)_2$ was administered i.g. at the 7 day LD_5 and LD_{25} levels. At these concentrations, the pH of the $Pt(SO_4)_2$ solutions was approximately 1.0. To compensate for the effects of low pH, a low pH control, containing no Pt was included in the study. The sulfate concentration and pH of this solution were identical to those of the LD_{25} dose. To compensate for effects of handling and administration, a saline control also was included.

The animals received either: (i) 144 mg Pt/kg (LD_5); (ii) 213 mg Pt/kg (LD_{25}); (iii) 0.25 M sulfuric acid adjusted to pH 0.85 (pH of LD_{25} solution) with sodium hydroxide or (iv) 0.14 M sodium chloride. In each case the volume administered was equal to 1% (on a volume/weight basis) of body weight.

Protocol

Immediately after observation the animals were sacrificed by overdosing with ether and dissected. Blood was obtained by cardiac puncture and the liver, spleen, kidneys, lungs, testes, skeletal muscle, cerebrum, cerebellum and medulla were removed, weighed and placed in acid washed test tubes.

Tissue Pt levels were analyzed employing a modification of the method of Tillery and Johnson (7). Only tissues from Pt exposed animals were analyzed. (A pilot study showed the tissue levels of animals not receiving Pt to be below the detection limit of the assay). Tissues of the Pt group animals were analyzed for Pt by flameless atomic absorption spectroscopy employing a wet ashing procedure. Samples were collected in acid washed test tubes. Digestion proceeded as follows: 5 ml conc. nitric acid was added and the samples were heated at 70°C until the nitric acid boiled off; 5 ml conc. HCl was added and boiled off; 2 ml 70% perchloric acid was added and heated to approx. 200°C to complete digestion. The perchloric acid was boiled off and a Pt-Sn complex was formed by the addition of a 2% stannous chloride

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solution in 3 N HCl. The negatively charged complex was extracted with two 0.5 ml aliquots of 20 mM tri-n-octylamine in xylene. Extraction was necessary to obtain Pt free of interfering inorganic salts present in the tissues. Platinum levels were measured with a Perkin-Elmer Model 360 atomic absorption spectrophotometer equipped with a Model HGA- 2100 graphite furnace. A 25 µl aliquot of the sample in xylene solution was placed in the graphite furnace. The sample was dried at 160°C, charred at 900°C and atomized at 2700°C. Samples were compared to a standard curve, included in each run, obtained from aqueous standards extracted into xylene in the same manner as the samples.

RESULTS

Tissue/organ Pt concentrations (for the Pt exposed groups only) were subjected to a multivariate analysis of variance (ANOVA) (8). There was a significant multivariate time effect with significant effects for all tissues except spleen (Table 3). The multivariate dose effect also was significant with significant effects for all tissues except brain. The multivariate time by dose interaction was significant; but only muscle and cerebellum had significant univariate effects.

The actual tissue/organ Pt concentrations are shown in Table 4. The nature of the time and dose effects are readily apparent. Platinum concentrations in the blood, kidney, lung, muscle and brain tissue are highest at 4 hours post administration. The liver and spleen Pt concentrations rise to a maximum at 3 days and decrease by 7 days. Testes Pt levels, however, are high initially, decrease 1 day post administration, and peak at 7 days. It is of interest to note that tissue Pt concentrations in the high dose groups were 3 to 10 times higher than in the low dose groups even though the high dose groups received only about 1.5 times as much Pt.

B. $Pt(SO_4)_2$-MULTIPLE (SERIALLY REPEATED) DOSE STUDY

MATERIALS AND METHODS

Animals

Six to 8 week old male mice served as subjects. Animals were randomly assigned to each of the 3 treatment groups (100 animals plus an allowance for possible deaths were each assigned to the $Pt(SO_4)_2$ and low pH groups while 50 animals plus an allowance were assigned to the saline control group).

Dosing

The experimental animals received 1 to 10 doses of Pt i.g. at the rate of 109 mg Pt/kg (equivalent to the 7 day i.g. LD_1 dose) in a volume equivalent to 1% (v/w) of body weight. Low pH controls received an equivalent volume of

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0.15 M H_2SO_4, adjusted to pH 1.18 with NaOH (which was identical in sulfate concentration and pH to the Pt solution). Saline controls received an equivalent volume of 0.14 M NaCl.

Protocol

Three days post administration, 10 animals from the Pt and low pH groups and 5 animals from the saline group were randomly selected for behavioral testing. The remaining animals were dosed again as described and this procedure was continued for a total of 10 dosings involving a total of 250 animals. Animals were always tested 3 days post administration.

After 2, 4, 6, 8 and 10 doses, the Pt group animals were sacrificed immediately after behavioral observation and a blood sample, the liver, kidneys, spleen, lung, G.I. tract, testes and brain were collected in acid washed test tubes. Tissue Pt concentrations were determined as described above.

RESULTS

The tissue Pt concentrations are presented in Table 5. Brain levels were uniformly undetectable (less than 0.15 µg Pt/g tissue) and, therefore, not analyzed. The multivariate ANOVA (8) revealed a significant effect of number of doses ($F = 2.14$, d.f. = 28/142, $p < 0.01$). The dose effect was also significant for blood ($F = 4.09$, d.f. = 4/45, $p < 0.01$), liver ($F = 4.29$, d.f. = 7/45, $p < 0.01$), kidney ($F = 3.55$, d.f. = 4/45, $p < 0.05$) and testes ($F = 5.80$, d.f. = 4/45, $p < 0.001$), and very nearly significant for spleen ($F = 2.56$, d.f. = 4/45, $p < 0.051$). In all cases, the effect was one of higher Pt concentrations at greater numbers of doses.

C. Na_2PtCl_6-SINGLE (ACUTE) DOSE TISSUE DISTRIBUTION STUDY

MATERIALS AND METHODS

Animals

One hundred and sixty 6-8 week old male mice served as subjects. The animals were randomly assigned to 16 treatment groups.

Dosing

Two Pt dose levels were employed, the 7 day i.g. LD_{25} and the 7 day i.g. LD_5 (69.3 mg Pt/kg and 54.0 mg Pt/kg, respectively) as determined statistically from the lethality study. The pH of the Pt solutions administered [in a volume equivalent to 1% (v/w) of body weight] was approximately 2.6. Since the pH of neutral Na_2PtCl_6 solutions is unstable, the Pt dosing solutions were not neutralized. It is recognized that pH affects toxicity. Thus, a low pH control group (3 mM HCl) was included in these studies to compensate

for the effects of low pH. In addition, a 30 mM NaCl solution which had approximately the same ionic strength as the LD_{25} solution, served as a control for effects related to administration. All solutions were administered i.g.

Protocol

At 4 hours or 1, 3 or 7 days post administration, the animals were observed individually for five minutes each in the open field and the exploratory apparatus as described below in the behavioral studies. Order of observation was counterbalanced. Immediately following observation, the animals were sacrificed by overdosing with ether and dissected. Blood was obtained by cardiac puncture and the liver, spleen, kidneys, cerebrum and brain stem were removed, weighed and analyzed for Pt [as described above in the $Pt(SO_4)_2$ study].

RESULTS

Tissue/organ Pt concentrations are presented in Figure 1. The results of the multivariate ANOVA (8) for tissue Pt levels are presented in Table 6. As can be seen, there was a significant time effect for blood, liver and kidney and a significant dose effect for spleen and kidney. The nature of these effects is apparent from Figure 1. In the case of the time effect, kidney levels peaked at four hours whereas liver levels peaked at one day and blood levels peaked between 4 hours and 1 day. In all 3 tissues, Pt levels declined from their maxima through 7 days post administration.

In the case of the treatment effect, the higher Pt dose (LD_{25}) produced significantly higher Pt levels in both the spleen and kidney.

Relative to the dose administered, the fairly low tissue/organ Pt levels observed suggest that Na_2PtCl_6 is only poorly absorbed from the gut. With the exception of the spleen, the biological half-life of Pt administered as Na_2PtCl_6 appears to be short.

D. Na_2PtCl_6-DEVELOPMENTAL STUDIES

MATERIALS AND METHODS

Animals

Females were maintained and bred as described above in the lethality study.

Dosing

On days 7 or 12 of gestation or day 2 post partum, the experimental animals received (s.c.) 72 ppm Pt (7 day LD_1) in a volume equivalent to 1% (v/w) of body weight. Controls received PBS only.

Protocol

Adult animals were sacrificed by cervical dislocation at times up to 5 days post Pt administration. Maternal tissues (blood, liver, kidney, spleen, and brain) and the embryo/fetus and placenta were removed for Pt analysis.

Offspring of dams receiving Pt on day 2 post partum were sacrificed by overexposure to ether vapor at times up to day 12 post partum. On days 3, 5 and 7 post partum, offspring whole body Pt content was determined. On day 12 post partum, selected offspring tissues [blood, liver, kidney, spleen, brain and digestive tract (stomach and small and large intestine)] were analyzed for Pt.

RESULTS

Whole embryo/fetal and placental and maternal tissue levels of Pt following maternal administration of Na_2PtCl_6 on day 7 of gestation are shown in Table 7. Blood, kidney and embryo Pt levels were highest 1 day post administration while maximal levels in liver and spleen were not reached until 3 days post administration. Platinum was undetectable in brain tissue. Maximal tissue Pt levels (in ppm) were: kidney (63.10 ± 2.80), liver (17.20 ± 5.30), spleen (11.70 ± 4.40), blood (8.70 ± 1.07) and embryo (5.56 ± 0.43). Analysis of fetal and placental tissues 5 days post administration detected Pt only in the placenta (at a level 2.5 fold greater than in maternal blood).

Maternal tissue, fetal and placental Pt levels following day 12 administration are shown in Table 8. All tissues showed maximal Pt levels 1 day post administration except for the spleen in which Pt levels increased throughout the 5 day period of analysis. The highest Pt levels (in ppm) were found in kidney (49.40 ± 1.30) followed by liver (22.40 ± 3.20), spleen (13.80 ± 3.10), blood (5.10 ± 0.89), placenta (6.90 ± 1.20), brain (0.43 ± 0.01) and fetus (0.22 ± 0.07). Both maternal brain tissue and the fetus accumulated low levels of Pt one day post administration; but Pt was detectable in neither during later stages of gestation. Placental tissue Pt levels were always greater than blood levels and reached concentration factors of 1.3 and 4.2 on days 1 and 5 post administration, respectively.

Platinum levels in the suckling offspring of dams administered Pt on day 2 post partum are shown in Table 9. One day post administration, Pt levels were 0.37 ± 0.08 and 0.24 ± 0.06 ppm in male and female offspring, respectively. These levels dropped 2.5 to 3.5 fold during the next 4 days. On day 12 post partum, individual offspring tissues were analyzed for Pt content. Except for the digestive tract (stomach; small and large intestine) and its contents, which contained Pt levels of 1.16 ± 0.15 and 1.01 ± 1.17 ppm (mean ± SEM for 5 animals) for males and females, respectively, no Pt was detectable.

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III. BEHAVIORAL STUDIES

Behavioral alterations resulting from exposure to acute and serially repeated doses of Pt (as $PtSO_4$ or Na_2PtCl_6), administered via the i.g. route, were investigated. These data were correlated with those of the corresponding tissue distribution study (above) in an attempt to gain insight into the mode of toxic action of the Pt compounds.

A. $Pt(SO_4)_2$-SINGLE (ACUTE) DOSE STUDIES

1. Open Field Behavior

MATERIALS AND METHODS

Animals

The same animals employed in the acute $Pt(SO_4)_2$ tissue distribution study described above served as subjects.

Dosing

Animals received (i.g.) either the 7 day LD_5 (144 mg Pt/kg) or LD_{25} (213 mg Pt/kg). Two control groups were employed: a low pH group which received 0.25 M H_2SO_4 adjusted to pH 0.85 (pH of LD_{25} solution) with NaOH and a saline group which received 0.14 M NaCl in a volume equal to 1% (v/w) body weight.

Apparatus

A Lehigh Valley Electronics (Fogelsville, PA) Model 1497 automated open field was employed.

Protocol

The subjects were observed in the open field at 4 hours or 1, 3, or 7 days post administration. Each subject was placed in the central circle (diameter = 15 cm) of the field and observed for 5 minutes. The dependent measures (which we view as providing an index of general activity) were total ambulations (automatically recorded) and total rearings (visually recorded). A rearing was scored each time the subject raised both forepaws off the floor in the absence of grooming behavior.

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RESULTS

The behavioral data (Figure 2) were analyzed (Table 10) using a two way, time of observation by treatment, multivariate ANOVA (8). The time effect was significant overall, and for ambulations, separately. The time effect for rearings approached significance. The treatment effect (which compares all subjects receiving each treatment with those receiving other treatments, regardless of the time of observation) was significant overall and for ambulations, separately. Again, rearings approached significance. The time by treatment effect [which tests for interactions of the two main effects (time and treatment) that are not simply additive] was not statistically significant.

To determine the basis of the time and treatment effects, the behavioral data (Figure 2) were subjected to Tukey's Honestly Significant Difference Test (9). The time effect for ambulations was found to be due to the 4 hour animals having a lower score than the 1 day animals. However, neither of these groups differed from the 3 or 7 day groups. The treatment effect for ambulations was due to the LD_{25} groups having lower scores than all other treatment groups (which did not differ among themselves). The effect only approached significance for rearings. However, the pattern for rearings parallels that for ambulations.

Relationship Between Open Field Behavior and Tissue Pt Levels: The correlations between behavioral performance and tissue/organ Pt concentrations are shown in Table 11. Again, the data base for this analysis was derived from the Pt exposed animals only since Pt concentrations were undetectable in control animals. The tests of no association are multivariate tests of whether the two sets of variables, behavior and tissue/organ Pt concentrations, are associated with one another. These tests revealed significant associations for both behavioral measures together and, also, separately.

Ambulations were significantly correlated with all tissues except brain. Rearings were correlated with all tissues except brain and muscle. Both ambulations and rearings had strong negative correlations with spleen, blood, testes, liver and lung (in that order) and a weaker correlation with kidney. The negative correlations indicate that the more depressed behavioral scores were associated with the higher tissue/organ Pt concentrations.

2. Exploratory Behavior

Effects of acute (single dose) exposure to $Pt(SO_4)_2$ on "hole-in-board" exploratory behavior of the adult mouse were investigated as a function of dose (LD_5 vs LD_{25}) and time of observation.

The "hole-in-board" task requires little physical movement on the part of the subject and, thus, provides a measure of exploratory drive which is not greatly confounded by general activity.

MATERIALS AND METHODS

Animals

One hundred and sixty adult male mice served as subjects. The composition of the experimental group was identical to that described above in the $Pt(SO_4)_2$ single dose tissue distribution study.

Dosing

$Pt(SO_4)_2$ was administered as described above in the single dose tissue distribution study.

Apparatus

The exploratory apparatus consisted of a circular runway, 5.7 cm wide, formed from two concentric walls with an O.D. of 34.3 cm. In the outside wall were four holes centered 90° apart and 5.1 cm above the floor. Photocells were arranged so that each time a subject placed its nose into a hole the event was counted. The runway was specifically designed to minimize the general activity component of this task and maximize the exploratory component.

Protocol

The subject (randomly selected) was placed in the runway and the number of hole entries, (automatically recorded) over the 5 minute observation period was the measure of exploratory behavior.

RESULTS

Significant effects of time ($F = 6.66$, d.f. = 3/144, $p < 0.001$) and time by treatment ($F = 1.92$, d.f. = 9/144, $p < 0.05$) were found. Tukey's Honestly Significant Difference Test (9) of the time effect showed exploratory behavior to be higher at 4 hours than all other times with no difference among the other times. The time by treatment interaction primarily appears to be due to the 4 hour low pH control group which explored significantly more than other 4 hour or low pH groups.

All groups were similar at 4 hours with the exception of the low pH group which manifested a significantly higher level of exploratory behavior. The balance of the interaction is taken up by the fact that exploratory behavior decreased at 1, 3 and 7 days for all groups except the saline control group whose behavior remained constant across time.

3. Activity Wheel Performance

This study was undertaken to investigate the effects of acute (single dose) exposure to $Pt(SO_4)_2$ on a measure of long-term general activity. These data complement those obtained from the open field study by providing a (relatively) long term index of effects on activity.

MATERIALS AND METHODS

Animals

Forty adult male mice (4 groups of 10, randomly selected) served as subjects.

Dosing

Two groups of experimental animals were investigated. One group of ten animals received a single i.g. dose of $Pt(SO_4)_2$ at the LD_5 level (144 ppm Pt), the other, a single LD_{25} dose (213 ppm). Two groups of control animals were employed. One received a single dose of 0.14 M NaCl, the other, a single dose of 0.25 M H_2SO_4 adjusted to pH 0.85 with NaOH.

Apparatus

The activity wheels with attached home cage [modeled after the standard Wahmann (LC34) mouse activity wheel] were constructed of plexiglass. The activity wheel was 20.0 cm in diameter and 6.8 cm in width. The dimensions of the home cage were 23.75 x 17.5 x 12.5 cm.

Protocol

Total wheel revolutions were recorded continuously during the dark phase of the light cycle (lights on at 7:00 p.m., off at 7:00 a.m.). Scores (total wheel revolutions) for each of 7 consecutive days post administration constituted the behavioral measure.

Two separate analyses were performed on the data. Total wheel revolutions at 4 hours post administration were subjected to a one-way ANOVA. This time was chosen in order to evaluate the short-term effects of Pt administration. In order to evaluate the long-term effects of Pt administration, total wheel revolution scores for each of the 7 days post administration were analyzed by a one-way multivariate ANOVA for repeated measures. The Pt exposure groups comprised 4 levels of the same factor (TREATMENT). Wheel revolution scores for each of the 7 days were treated as multiple intercorrelated responses from the same subjects (DAYS). To test the various effects, new variables were formed by combining the 7 original "day" scores. The treatment effect was obtained by summing the individual day scores and testing the resultant variable for differences among treatments. The trend analysis across time (8) was obtained by combining the original variables using orthogonal polynomial coefficients to obtain linear, quadratic and cubic combinations. These combinations were tested for differences from zero which occur only if that trend (linear, quadratic, cubic) is significant. The treatment x days interaction was obtained by testing the same combinations (linear, etc.) for differences among treatment groups. The trends were tested in a step down fashion which provides tests of each trend free of effects due to lower order trends (e.g. cubic effect free of linear and quadratic effects).

RESULTS

The one-way ANOVA of total wheel revolutions recorded at 4 hours post administration for the experimental and control groups failed to demonstrate differences between groups.

Analysis of activity through day 7 post administration revealed no significant overall effect of treatment (Pt vs controls) and no significant treatment by days interaction. The only significant effect was a quadratic trend ($F = 13.90$, d.f. = 7/35, $p < 0.001$) for the means (Figure 3) of all treatment groups.

4. Passive Avoidance Learning

To investigate effects of exposure to a single (acute) dose of $Pt(SO_4)_2$ on higher brain processes than may be predominately involved in general activity and exploratory behavior, passive avoidance, a measure of simple learning, was investigated. Since the passive avoidance paradigm provides a learning measure which requires little activity on the part of the subject (which, as we have shown, may be depressed by exposure to Pt), it should provide a measure of learning that is not confounded extensively by effects on activity level.

MATERIALS AND METHODS

Animals

The same animals used in the single (acute) dose exploratory behavior study described above were used in this study.

Dosing

$Pt(SO_4)_2$ was administered as described in the single (acute) dose exploratory behavior study.

Apparatus

The passive avoidance apparatus consisted of a larger and smaller compartment separated by a (2.5 cm) hurdle. The interior of the smaller compartment (5.1 x 17.0 cm) was painted white, that of the larger (17.8 cm x 20.3 cm) compartment, black. Both compartments had a grid floor. That of the larger was wired to receive a scrambled shock.

Protocol

Each subject was placed into the smaller compartment and the time taken to cross into the larger compartment was recorded (trial 1 latency). One second after entering the larger (fear) compartment the subject was given a 0.1 mA shock which lasted until the subject crossed back into the smaller (safe) compartment. Twenty-four hours later (to allow for learning consolidation), the subject was again placed into the smaller compartment

and the time required to cross into the larger compartment was recorded (trial 2 latency). A significant difference between trial 1 and trial 2 latencies (trial 2 being longer) represents significant learning.

The experiment was analyzed as a two between (time, dose), one within (trials) repeated measures design using the sum of the two trials to test the between effects and the difference between trials to test the within effects (all effects involving trials).

RESULTS

No effects either of time or treatment were observed. The only significant effect found was that of trials. This was due to increased trial 2 latency ($F = 21.70$, d.f. = 1/44, $p < 0.001$) indicating learning in all animals.

5. Active Avoidance Learning

Effects of exposure to a single (acute) dose of $Pt(SO_4)_2$ at the LD_5 or LD_{25} levels, on two-way active avoidance, a measure of complex learning, were investigated. Two-way active avoidance is a well established measure of learning ability for a variety of species including pigeons, rodents and cats (10,11). In this procedure, a conditioned avoidance response is developed in a situation in which a signal (of 10 seconds duration) is associated with electric shock from which the animal can escape by moving from the "fear" compartment to the "safe" compartment. By learning to move as soon as the signal is initiated, the animal can avoid the shock. Avoidance learning accepted as a sensitive measure of learning and has been used to study the effects of drugs on learning (12).

MATERIALS AND METHODS

Animals

Forty 6-8 week old male mice served as subjects.

Dosing

The subjects were divided randomly into 4 groups of 10 and dosed i.g. The experimental animals received $Pt(SO_4)_2$ either at the 7 day LD_5 (144 mg Pt/kg or LD_{25} (213 mg Pt/kg) levels. Two control groups were employed: a low pH group, which received 0.25 M H_2SO_4 adjusted to pH 0.85 (pH of LD_{25} solution) with NaOH, and a saline group, which received 0.14 N NaCl. All doses were delivered in a volume equal to 1% (v/w) body weight. All subjects were lightly etherized to facilitate i.g. administration.

Apparatus

An automated two-way active avoidance box was used. The box was divided into 2 compartments by a center doorway. A shock grid comprised the floor of each compartment. The grids were made of stainless steel rods. Photocells were located on either side of the doorway to record crossing of the

subject between the 2 compartments. The conditioned stimulus (CS) was a 1000 Hz tone from speakers located at each end of the apparatus. The unconditioned stimulus (UCS) was a 0.2 mA scrambled shock. The CS-UCS interval was ten seconds. The inter-trial intervals varied from 30 to 60 seconds with a mean of 45 seconds. Subjects could avoid the shock by moving to the opposite compartment during the 10 second CS-UCS interval. If such a response was made, the CS was terminated and the shock was withheld. If the subject did not cross during the 10 second interval, the shock was initiated and was terminated when the subject crossed into the opposite compartment. Crossings were allowed at all times. Solid-state programming equipment recorded avoidance responses, escape responses and all crossings.

Protocol

Training began 24 hours post-administration. All subjects received 25 trials per day for 5 consecutive days or until they reached a learning criterion of 9 out of 10 avoidance responses. The measure of trials to criterion was the dependent learning variable. An activity score was calculated for each subject by dividing the total spontaneous crosses by the trials to criterion.

RESULTS

Trials to criterion and activity scores were analyzed by separate one-way ANOVAs. Effects of exposure to $Pt(SO_4)_2$ on trials to criterion and activity scores were not significant ($F = 0.67$, d.f. = 3/36; $F = 0.14$, d.f. = 3/36).

6. Social Behavior

Covert toxic reactions may be manifested as subtle alterations of complex behavioral patterns. Thus, the effects of Pt on 3 measures of murine social behavior were investigated.

MATERIALS AND METHODS

Animals

One hundred and sixty 6-8 week old male mice served as subjects.

Dosing

The dosing conditions were identical to those described above in the active avoidance learning study, except there were 40 animals in each dose condition.

Apparatus

The apparatus consisted of a circular open field, 1.2 m in diameter, enclosed by a 46.0 cm high wall. The field was painted white and was divided into 49 sections of equal shape and area by a series of black concentric circles and radii. Each section was numbered (from 1 to 49).

Protocol

The treatment groups were observed at 1 day or 3 days post administration. Thus, a 4 (dose) by 2 (time of observation) experimental design was employed. This design created 6 conditions with 20 subjects (selected at random) per condition.

Animals were paired randomly within a given condition creating 10 pairs per cell. The pairs were observed in the open field for a 5 minute period. The number of pair contacts and the total time in contact were measured as 2 indicators of social attraction. Also, the location of each animal was recorded photographically at 10 second intervals. From the film record, the mean distance apart maintained by the subjects was calculated and served as an index of social attraction. Finally, from the film record, a mobility (or activity) score was computed for each subject based upon the straight line distance between the numbered sections occupied by the subject in consecutive 10 second intervals.

RESULTS

A multivariate ANOVA (8) revealed no significant differences among groups for either the main effects of dosage ($F = 1.61$, d.f. = 6/142, $p < 0.15$), time of observation ($F = 2.75$, d.f. = 2/71, $p < 0.07$) or the dosage by time of observation interaction ($F = 1.51$, d.f. = 6/142, $p < 0.18$). Since the multivariate analysis did not attain statistical significance univariate analyses were not undertaken.

B. Na_2PtCl_6-SINGLE (ACUTE) DOSE BEHAVIORAL STUDIES

1. Open Field Study

MATERIALS AND METHODS

Animals

The same animals as in the Na_2PtCl_6 tissue distribution study described above were employed as subjects.

Dosing

Na_2PtCl_6 was administered at the LD_5 or LD_{25} levels as described above in the tissue distribution study.

Apparatus

The apparatus previously described in the single (acute) dose $Pt(SO_4)_2$ open field study was used.

Protocol

The experimental procedures were as described above in the single (acute) dose $Pt(SO_4)_2$ study. Data were analyzed in a two way (time of observation by treatment) multivariate ANOVA (8).

RESULTS

The results of the ANOVA are presented in Table 12 and the open field data are presented graphically in Figure 4.

There was a significant multivariate time effect with a significant univariate effect for ambulations. The treatment effect was highly significant in the multivariate test and for ambulations and rearings separately. The time by treatment interaction also was significant in the multivariate and univariate tests. Tukey's Honestly Significant Difference Test (8) revealed that the time effect was due to the greater number of ambulations across treatments exhibited by the 4 hour and 1 day groups compared to the 3 and 7 day groups. The treatment effect for ambulations was due to a lower score for the LD_{25} group than the low pH or saline groups. For rearings, both Pt groups had lower scores compared to the saline group but not the low pH group. The interaction for ambulations was due to a lower score for the LD_{25} group than all other groups at 4 hours; but not at any other time. For rearings, the LD_{25} group had a lower score than both control groups at 4 hours and a lower score than the saline group at three days. The LD_5 group had a lower score than the saline controls at 1 and 3 days. No other differences were found. The depressant effect of Na_2PtCl_6 on ambulations and rearings in the open field was statistically distinguishable from the effects of low pH alone only at 4 hours post administration. It is possible that the time effects and the interaction may be artifactual since the time groups were run as blocks on different days and were not randomized. Thus, shifts in instrument parameters or environmental conditions could confound these effects. However, the order of observation of subjects from the various treatments was randomized and equal numbers from each treatment were observed each calendar day. Therefore, the treatment effects were not similarly confounded.

2. Exploratory Behavior

MATERIALS AND METHODS

Animals

The same animals used in the Na_2PtCl_6 open field study described above served as subjects in this experiment.

Dosing

Experimental and control animals were treated as described above in the Na_2PtCl_6 tissue distribution study.

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Apparatus

The apparatus described in the comparable $Pt(SO_4)_2$ study was used.

Protocol

The experiment was conducted as described above in the comparable $Pt(SO_4)_2$ study.

Since this study was added after the 4 hour open field behavioral study had been completed, it contains only 3 times of observation instead of 4. Therefore, the data were analyzed in a separate univariate ANOVA.

RESULTS

Analysis of the data revealed no significant effects of the experimental treatments.

Relationship between open field and exploratory behavior and tissue Pt levels in Na_2PtCl_6 exposed animals: A significant overall association between tissue/organ Pt levels and ambulations and rearings was found (test of hypothesis of no association: $F = 2.23$, d.f. = 8/144, $p < 0.03$). However, neither ambulations nor rearings separately had significant associations with the tissue/organ Pt levels (ambulations: $F = 2.04$, d.f. = 4/73, $p < 0.10$; rearings: $F = 1.11$, d.f. = 4/73, $p < 0.36$). The only significant individual correlation coefficient was between ambulations and spleen ($r = -0.276$, d.f. = 78, $p < 0.05$). There was no significant association between tissue/organ Pt levels and explorations ($F = 1.00$, d.f. = 4/44, N.S.).

C. $Pt(SO_4)_2$-MULTIPLE (SERIALLY REPEATED) DOSE STUDIES

1. Open Field and Exploratory Behavior

MATERIALS AND METHODS

Animals

The same animals used in the $Pt(SO_4)_2$ repeated dose tissue distribution study described above served as subjects in this study.

Dosing

The animals were dosed as described above in the $Pt(SO_4)_2$ repeated dose tissue distribution study, generating three treatment groups: $Pt(SO_4)_2$, low pH control and saline control.

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Apparatus

The apparatuses used in this study were the same as those described above in the acute $Pt(SO_4)_2$ open field and exploratory behavior studies.

Protocol

The experiments were conducted as described in the comparable $Pt(SO_4)_2$ studies. Data were analyzed by multivariate and univariate ANOVA (Number of Doses x Treatment).

RESULTS

The means (± SD) for the three behavioral measures (ambulations, rearings and explorations) are presented in Table 13. Multivariate ANOVA of these data (Table 14) revealed a significant effect of number of doses with rearings as the only significant univariate measure. The treatment effect approached significance ($p < 0.07$) with significant univariate F-ratios for ambulations and explorations. The doses by treatment interaction also approached significance with a significant univariate effect for rearings and with explorations approaching significance. Tukey's Honestly Significant Difference Test (9) revealed that, at doses 7, 8, 9 and 10, rearings were decreased more than at dose 2 and at doses 7 and 8, rearings were decreased more than at dose 3. Tukey's procedure is not entirely efficient for unequal cell frequencies and failed to find any pair-wise differences among treatments for ambulations and explorations despite significant overall F-ratios. Therefore, linear combinations of the treatments (Pt vs low pH and saline; low pH vs saline) were tested as t-statistics to locate the effect. Ambulations and explorations were found to be depressed in the Pt exposed groups relative to the control groups by this procedure while the control groups did not differ. The time by treatment interaction for rearings arose because, at times 7 and 10, scores for the Pt exposed groups were lower than those for the saline control groups. The time by treatment interaction for explorations arose because at time 10 only, the scores of the Pt exposed groups were lower than those of both control groups.

Relationships between behavior (open field and exploratory) and tissue Pt levels: Overall tests of association and correlation between the behavioral measures and tissue Pt levels for the Pt treated groups observed after 2, 4, 6, 8 and 10 doses are presented in Table 15. A significant overall association was found. However, the only individual behavioral measure significantly associated with tissue Pt levels was rearings. Inverse correlations were found between rearings and liver, testes, kidney, spleen and blood Pt levels. They apparently reflect the decrease in rearings accompanying the increased tissue Pt levels that occurred with the increase in number of doses.

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D. DEVELOPMENTAL STUDIES

1. Effect of $Pt(SO_4)_2$ on Offspring Weight and Selected Maternal, Neonatal and Adult Offspring Behaviors

Although it is well known that the developing mammalian central nervous system (CNS) is particularly vulnerable to insult from environmental toxicants, essentially nothing is known about the effects of Pt (compounds) on mammalian development, growth and behavior. Therefore, we undertook an investigation of effects of $Pt(SO_4)_2$ on the murine maternal/offspring unit and offspring development and behavior. Our experiments were designed to investigate relatively long-term effects of Pt administered at specific times during gestation and lactation on: (i) selected postnatal offspring parameters (weight, general activity of the neonate, open field behavior and simple learning), and (ii) maternal behavior (offspring retrieval latency).

MATERIALS AND METHODS

Animals

The animals were selected and maintained as described above in the $PT(SO_4)_2$ tissue distribution study. Females were bred daily to competent males between 9:00 AM and 2:00 PM. The occurrence of a copulatory plug was recorded as Day 0 of gestation.

Dosing

On days 7 or 12 of gestation (gestation groups) or on day 1 post partum (lactation group), females received (i.g.) $Pt(SO_4)_2$ at the gravid 7 day LD_1 level (200 mg Pt/kg). Two control groups were employed: a low pH group receiving 0.25 M H_2SO_4 adjusted to pH 0.85 with NaOH and a saline group receiving 0.14 N NaCl in a volume equal to 1% (v/w) of body weight. Data were collected on 2 control groups because of a concern that the low pH solution itself might produce behavioral changes. However, the 2 controls did not differ. Therefore, the experimental group was contrasted with the low ph control only for ease of interpretation. The mice were lightly etherized to facilitate intubation.

Apparatus

The activity field employed for investigating the general activity of 7 day old neonates is described in Figure 5. The circular runway employed for investigating the general activity of 11 day old neonates is described in Figure 6. The apparatuses employed for investigating adult offspring behavior in the open field and passive avoidance learning have been described (above) in comparable experiments on adult animals exposed to $Pt(SO_4)_2$. Adult rotarod performance was examined employing an accelerating rotarod. This apparatus consists of a steel rod, 46.0 cm long and 1.9 cm in diameter, covered with rubber tubing for traction. The covered rod has a diameter of 3.8 cm. The rod is divided into five 7.6 cm compartments by masonite discs (25.0 cm in

diameter). Each end of the bar is run on a ball race. The rod is coupled via a 100:1 reduction gearbox to a permanent magnet DC motor connected in series to a 100 ohm torgoidally wound resistor. This resistor controls a kymograph motor so that a change in speed from 2 rev/min (maximum resistance) to 50 rev/min (minimum resistance) is obtained over a period of 5.5 min. To serve as an arbitrary measure of the speed of rotation at which a subject was driven off the rod, a gear wheel was divided into 10 equal sectors, numbered 1 to 10 (maximum to minimum resistance).

Protocol

On the day of parturition (day 0), all litters were culled to 3 males and 3 females. For the offspring of the gestation groups, a complete cross-fostering design was employed. [There were 4 cross-foster groups: (1) in utero Pt exposure x postnatal Pt exposure (via suckling a Pt exposed foster mother); (2) in utero Pt exposure x postnatal exposure to low pH (via suckling a low pH exposed foster mother); (3) in utero exposure to low pH x postnatal Pt exposure; (4) in utero exposure to low pH x postnatal exposure to low pH].

In the lactation group, pups were randomly assigned to foster mothers on the day of parturition.

Neonates: Neonatal activity was monitored on days 8 and 11 post partum. The measure of general activity was the number of lines crossed during one minute of observation on the activity board (8 day old neonates only) or circular runway (11 day old neonates only). Each animal was tested once between 8:00 a.m. and 11:00 a.m. (during the dark phase of the light cycle: light on at 11:00 a.m., off at 11:00 p.m.). The animals were weighed immediately prior to testing and these weights comprised the measure of growth.

Adult offspring: Effects of exposure to Pt in utero or during sucking on ambulations and rearings in the open field (general activity), rotarod performance (balance and coordination) and passive avoidance (simple learning) were monitored on day 45 post partum. All animals were tested on all 3 tasks in the order: open field behavior, rotarod performance and passive avoidance learning. Rotarod performance was investigated by placing either 2 males or 2 females from each litter on the stationary rod. The measure of performance was the relative speed of rotation at the time the subject dropped off the rod. Each subject was given two trials; the second trial coming 30 minutes after the first. The resistor was not set back to maximum resistance after each trial so that the score for each trial was not a reflection of an individual subject's ability to improve its performance on succeeding trials.

The open field and passive avoidance protocols were as described above in the studies of behavioral effects of exposure of the adult mouse to $Pt(SO_4)_2$.

All tests were performed between 8:00 a.m. and 11:00 a.m. The animals were weighed immediately prior to testing on the first task and their weights comprised the measure of growth.

Maternal behavior: Maternal offspring retrieval latency was measured on day 3 post partum during the 1st hour of the light phase of the light cycle. Mothers (at least 5) from each of the 4 cross-fostering conditions were tested. The standard litter scatter technique was employed: the mother was removed to a holding cage, the litter was removed and individual pups were replaced in a randomly scattered manner in an area furthest from the maternal nest. Retrieval latency was measured as the time between replacement of the mother into the nest and the transport of the first pup to the nest. Incomplete retrievals were noted.

RESULTS

The behavioral performance and growth of offspring were analyzed using a 2 (prenatal exposure: biological mother receiving Pt or H_2SO_4) by 2 (postnatal exposure: foster mother receiving Pt or H_2SO_4) by 2 (sex of offspring) factorial ANOVA design. Neonate and adult parameters were analyzed separately for gestational day of Pt administration (day 7 or day 12). Data were analyzed using the Multivariance Computer Program, Version 6.1 of Finn (8).

a. Day 7 Gestational exposure, neonatal, adult offspring and maternal behavioral effects: Significant multivariate effects of prenatal exposure were found for offspring of mothers receiving $Pt(SO_4)_2$ on day 7 of gestation ($F = 2.66$, d.f. = 4/42, $p < 0.04$). Effects approaching significance were found for adult offspring measures. The significant univariate effect underlying the multivariate neonate effects was neonatal weight recorded on day 0 ($F = 8.01$, d.f. = 1/45, $p < 0.003$) and day 8 ($F = 6.64$, d.f. = 1/45, $p < 0.02$) post partum with the Pt exposed animals weighing less than the low pH exposed groups.

Analysis of the adult offspring measures for the day 7 gestational exposure groups showed a nearly significant multivariate effect of postnatal exposure ($F = 2.21$, d.f. = 7/46, $p < 0.05$). The underlying significant univariate effect was day 45 post partum weight ($F = 6.38$, d.f. = 1/52, $p < 0.02$) with offspring reared by Pt exposed mothers weighing less than those reared by low pH exposed mothers.

In the lactational study, there was a significant multivariate effect of maternal treatment ($F = 3.63$, d.f. = 5/32, $p < 0.01$). Further analysis found that the univariate effects underlying this multivariate effect were for neonatal day 0 weight ($F = 4.14$, d.f. = 1/36, $p < 0.05$) and day 11 post partum activity ($F = 8.48$, d.f. = 1/36, $p < 0.01$) with pups reared by mothers who had received Pt being less active than pups reared by low pH exposed control mothers. The day 0 weight difference must be regarded as being due to random error variance since the pups had not been exposed to any treatment in utero and assignment of pups to mothers was random and occurred before the mothers were treated.

b. Day 12 gestational exposure; neonatal, adult offspring and maternal behavior effects: Analysis of the neonatal measures for the day 12 gestational exposure groups revealed a significant multivariate effect of prenatal exposure ($F = 7.12$, d.f. = 4/45, $p < 0.001$) and a significant prenatal by postnatal exposure interaction ($F = 5.05$, d.f. = 4/45, $p < 0.002$). The significant univariate effect underlying the main effect for prenatal exposure was weight recorded on day 0 ($F = 25.12$, d.f. = 1/48, $p < 0.001$) and day 8 ($F = 6.99$, d.f. = 1/48, $p < 0.01$) post partum. In both cases, Pt exposed pups weighed less than low pH exposed pups. The univariate effect underlying the prenatal by postnatal interaction was weight on day 8 post partum ($F = 4.05$, d.f. = 1/48, $p < 0.05$).

Analysis of the adult offspring measures for the day 12 gestational exposure groups revealed a significant multivariate prenatal exposure effect ($F = 2.41$, d.f. = 7/43, $p < 0.04$). Further analysis of the multivariate effect revealed an underlying univariate effect on day 45 weight ($F = 10.66$, d.f. = 1/49, $p < 0.002$) with the Pt exposed offspring weighing less than the low pH exposed offspring. A marginal effect on rotarod trial 2 latencies ($F = 3.71$, d.f. = 1/49, $p < 0.06$) also was observed with the Pt exposed animals having lower scores than the low pH exposed animals.

2. Effects of Na_2PtCl_6 on Offspring Weight and Selected Maternal, Neonatal and Adult Offspring Behaviors

MATERIALS AND METHODS

Animals

Animal selection, maintenance and breeding were accomplished as described above in the comparable $Pt(SO_4)_2$ study.

Dosing

On days 7 or 12 of gestation or on day 1 post partum, females received Na_2PtCl_6 (s.c.) at the LD_1 level (20 ppm Pt in PBS). Controls received an equivalent volume (0.2 ml/30 gm) of PBS.

Protocol

Culling, cross-fostering and behavioral testing were accomplished as described above in the comparable $Pt(SO_4)_2$ developmental study.

The data were analyzed employing the same design described above for the comparable $Pt(SO_4)_2$ study.

RESULTS

a. Day 7 gestational exposure; neonatal, adult offspring and maternal behavior effects: No significant multivariate effects of in utero exposure were found for neonatal or adult offspring of mothers exposed to Na_2PtCl_6 on day 7 of gestation.

b. Day 12 gestational exposure; neonatal, adult offspring and maternal behavior effects: Analysis of the neonate scores of the day 12 gestational exposure groups revealed a significant multivariate effect of prenatal exposure ($F = 2.59$, d.f. = 4/52, $p < 0.05$). The significant univariate effect underlying this multivariate effect was for day 8 post partum activity ($F = 4.14$, d.f. = 1/55, $p < 0.05$) with Pt exposed pups being less active than control (PBS) pups.

Analysis of the adult offspring scores for the day 12 gestational exposure groups revealed a significant multivariate interaction of prenatal x postnatal exposure. The significant underlying univariate effects were: (i) ambulations ($F = 10.82$, d.f. = 1/55, $p < 0.002$); (ii) passive avoidance trial 1 latency ($F = 13.14$, d.f. = 1/55, $p < 0.01$); and (iii) passive avoidance difference (Trial 2 - Trial 1) scores ($F = 4.30$, d.f. = 1/55, $p < 0.04$). Simple tests (t-tests) showed that: (i) control pups fostered to PBS mothers had significantly more ambulations in the open field than did all other groups; (ii) the Pt x Pt group had longer passive avoidance trial 1 latencies than did the Pt x PBS group; and (iii) the Pt x Pt group had longer passive avoidance difference scores than did the Pt x PBS group.

No significant multivariate effect of suckling exposure were found for neonatal or adult offspring of mothers exposed to Na_2PtCl_6 on day 1 post partum.

IV. CONCLUSIONS

1. $Pt(SO_4)_2$-Single (Acute) Dose Tissue Distribution and Open Field Studies

In 1975, Moore et al. (13) reported on the biological fate of single doses of radiolabeled $PtCl_4$ in the rat. They observed that most of perorally administered Pt was not absorbed; being eliminated in the feces. We cannot confirm this finding directly, since feces were not collected in our study. However, the tissue Pt levels found in our study were low, representing only a small fraction of the administered dose. This is consistent with the findings of Moore et al.

Presently, we have no data to explain the disproportionately high tissue concentrations of Pt in animals receiving $Pt(SO_4)_2$ i.g. at the LD_{25} level as compared to the LD_5 level. Conceivably, the higher Pt dose may perturb the GI tract in some manner, resulting in facilitated Pt absorption. The high Pt concentrations found in the blood of such animals may damage the kidneys (which normally eliminate Pt quite efficiently), thereby decreasing Pt excretion and increasing tissue uptake. In addition, it is well known that some metals, such as cadmium (14) and lead (15), are able to induce the synthesis of their own binding proteins. Platinum may have a similar ability.

The ANOVA of the open field behavioral measures (Table 10) revealed that the higher Pt dose depressed ambulations significantly and rearings marginally, relative to other treatments, from 4 hours through 7 days post administration. A correlation analysis (Table 11) revealed highly significant negative correlations between behavioral measures and tissue levels of Pt in all tissues except brain and muscle. Since brain Pt levels were not related to behavior, it is not clear how non-CNS tissue Pt levels affect behavior. Waters et al. (9), working with alveolar macrophages in tissue culture, reported a 50% loss of viability (ability to exclude trypan blue) after a 20 hour exposure to 300 mM $PtCl_4$. Protein and RNA synthesis were inhibited by 50% by 60 mM Pt and DNA synthesis was inhibited by 50% by 10 mM Pt. The maximum tissue Pt concentration observed in our study was 50 ppm in the kidney, 4 hours post administration. Fifty ppm is approximately equivalent to 250 mM Pt. Therefore, cellular processes may have been disrupted to some extent in the LD_{25} animals which, in turn, may produce a general malaise and depression of the activity component of certain behaviors.

2. $Pt(SO_4)_2$-Single (Acute) Dose Effects on Exploratory Behavior

The high rate of explorations of the 4 hour low pH control group, for which no satisfactory explanation is presently available may have sufficiently accentuated the difference between the 4 hour time point and all other times of observation to account for the finding of a significant main effect of time as well as the significant time by treatment interaction.

At 1, 3 and 7 days post i.g. administration, the mean number of explorations for the 2 Pt groups (LD_5, LD_{25}) and the low pH control group was lower than that of the saline control group, but not significantly. Possibly, this trend was due to some nonspecific effect of the low pH or sulfate content of the solutions. In any case, it is apparently not a specific Pt effect. Thus, in this study, Pt exposure per se, does not appear to have effected changes in exploratory behavior.

3. $Pt(SO_4)_2$-Single (Acute) Dose Effects on Activity Wheel Performance

Platinum at either the LD_5 or LD_{25} levels appeared not to affect wheel running scores (Figure 3). All groups exhibited the lowest scores during the first 24 hours post administration. This may have resulted from trauma induced by handling and dose administration. The relative depression of scores observed on the last 2 days of testing may have been due to the effect of time (habituation). Trend analysis demonstrated that the performance curves of all treatment groups followed the same trend, independent of treatment. Thus, there were no overt effects of Pt exposure on activity wheel performance.

4. $Pt(SO_4)_2$-Single (Acute) Dose Effects on Passive Avoidance Learning

Intragastric administration of $Pt(SO_4)_2$ at the LD_5 or LD_{25} levels had no observable effect on passive avoidance learning by the adult mouse.

5. $Pt(SO_4)_2$-Single (Acute) Dose Effects on Active Avoidance Learning

Platinum sulfate at the LD_5 or LD_{25} levels had no effect either on the acquisition of two-way active avoidance learning or activity in the apparatus.

6. $Pt(SO_4)_2$-Single (Acute) Dose Effects on Social Behavior

At the i.g. LD_5 or LD_{25} levels, $Pt(SO_4)_2$ had no affect on the measure of murine social behavior investigated.

7. Na_2PtCl_6-Single (Acute) Dose Tissue Distribution and Open Field and Exploratory Behavioral Studies

Relative to the dose administered, the fairly low tissue/organ Pt levels observed suggest that Na_2PtCl_6 is only poorly absorbed from the gut. With the exception of the spleen, the biological half-life of Pt administered as Na_2PtCl_6 appears to be short.

Na_2PtCl_6 depressed ambulations and rearings in the open field. However, the effect was transitory and clear-cut effects were found only at 4 hours post administration (Figure 4). At 1 and 3 days post administration, the LD_5 exposed groups had depressed rearings relative to the saline controls groups but not the low pH control groups (Figure 4).

Na_2PtCl_6 had no observable effect on exploratory behavior at 1, 3 and 7 days post administration. Unfortunately, exploratory behavior was not examined 4 hours post administration when the strongest depression of open field behavior had been observed.

8. $Pt(SO_4)_2$-Multiple (Serially Repeated) Dose Effects on Tissue Distribution and Open Field Behavior

This study was undertaken to gain insight into the effects of subchronic exposure to Pt on certain behaviors of the mouse. Subchronic exposure was simulated via the use of a limited repeated dose experimental design (1 to 10 doses). Adult male mice were subjected to repeated i.g. administration (every 72 hours) of relatively high doses of $Pt(SO_4)_2$ (each dose equaled the 7 day LD_1, which is approximately 40% of the 7 day LD_{50}). This design resulted only in marginal adverse effects on the general activity and exploratory behavior of the adult animal. Tissue/organ Pt levels (Table 5), while highly variable, generally increased with the number of doses except for the brain in which no Pt was detected in any animal. The absence of Pt in the brain is not surprising since the highly charged Pt cation should be excluded by the blood brain barrier. Of the three behavioral measures studied, only rearings in the open field showed a significant correlation with tissue Pt levels (Table 15). Since this correlation involved a number of tissues and not the brain, a general systemic effect, rather than a neurotoxic effect, seems to be indicated. Inorganic Pt compounds have been shown to inhibit DNA, RNA and protein synthesis in tissue culture (16). Thus, it may be that Pt has a depressive effect on cellular metabolic processes and exposure to Pt above a threshold level results in general malaise which is reflected in the slight depression in behavior observed in this study.

9. $Pt(SO_4)_2$ Developmental Studies

The predominant effect of maternal administration of $Pt(SO_4)_2$ on days 7 and 12 of gestation was on offspring weight. The *in utero* Pt exposed offspring were lighter than low pH exposed offspring. This effect was powerful, beginning on day 0 and continuing through day 45 *post partum*. As indicated by the significant multivariate effect for postnatal exposure, type of foster mother exposure also has a significant effect on offspring weight. Thus, on day 45 *post partum*, pups (exposed to Pt or low pH *in utero*) reared by Pt exposed foster mothers weighed less than Pt or low pH gestationally exposed pups reared by low pH exposed mothers. The reason for the weight differences is unknown. However, it may be that reduced weight could have important consequences, such as reduced viability.

Administration of $Pt(SO_4)_2$ to lactating mothers resulted in symptoms of toxicity in offspring. Offspring assigned to mothers administered Pt during lactation exhibited depressed neonatal activity ($p < 0.006$) and lower adult weights ($p < 0.015$) than controls. Pt may have affected these offspring directly through the maternal milk supply and/or indirectly by affecting some aspect of maternal behavior.

10. Na_2PtCl_6 Developmental Tissue Distribution and Behavioral Studies

Maternal blood, kidney, and whole embryo Pt levels were highest 1 day post administration of Na_2PtCl_6 on day 7 of gestation. No Pt was detected in the maternal brain. By day 5 post administration, Pt was undetectable in fetal tissues, but the placental level was 2.5 fold greater than that of maternal blood.

Except for the maternal spleen, the Pt levels of maternal, fetal, and placental tissues peaked on day 1 post administration of Na_2PtCl_6 on day 12 of gestation. A low level of Pt (0.43 ± 0.01 ppm) was detected in maternal brain tissue. Placental Pt levels were greater than blood levels throughout the course of the experiment, reaching concentration factors, compared to blood, of 1.3 and 4.2 on days 1 and 5 post administration, respectively.

Whole body Pt levels in the suckling offspring of dams receiving Pt on day 2 *post partum* were highest on day 1 post administration. By day 12 post administration, except for the digestive tract and its contents, no Pt was detectable in the tissues of the sucklings.

Effects of maternally administered Na_2PtCl_6 on neonatal and adult offspring behavior were limited to day 12 gestational exposure. The neonatal effects were expressed as lower day 8 *post partum* activity scores for *in utero* Pt exposure compared to PBS exposure. For the adult offspring, there was a prenatal x postnatal exposure interaction indicating that Pt exposed mothers reduced the performance of offspring on those tasks with a large activity component (i.e., ambulations, passive avoidance trial 1 latency and passive avoidance difference scores). In this case, gestational exposure to Pt appears to have persistant effects on the mother that are transmitted postnatally to offspring. This may occur directly through residual Pt in the milk or indirectly through maternal behavioral alteration resulting in neglect of the offspring [manifested, for example, as ineffective suckling or lack of grooming (17)].

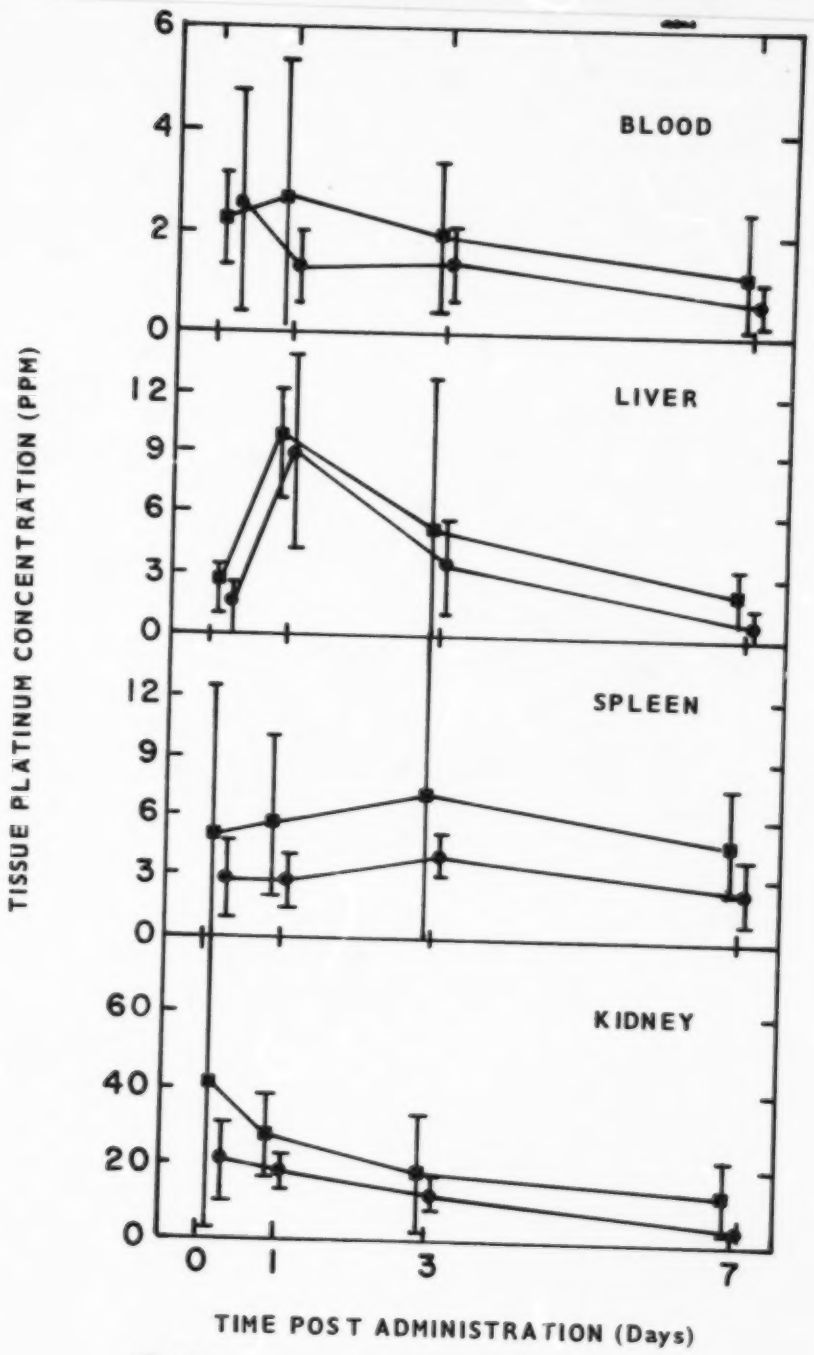


Figure 1. Time-dependent tissue/organ Pt concentrations (mg Pt/g wet weight tissue) following administration of a single (acute) dose of Na_2PtCl_6 to the mouse at the LD_5 (54.0 mg Pt/kg) and LD_{25} (69.3 mg Pt/kg) levels. Na_2PtCl_6 was administered intragastrically via intubation.

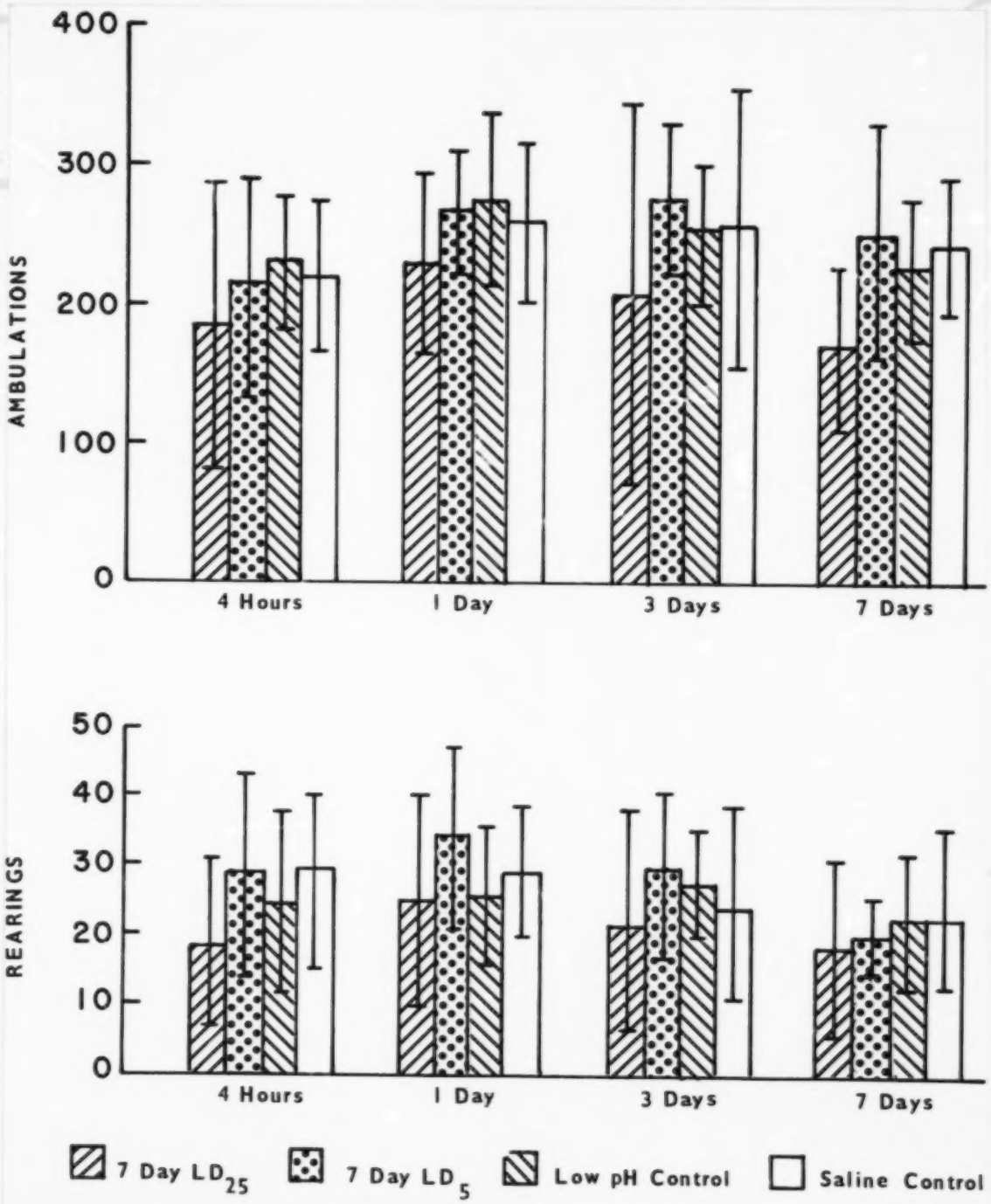


Figure 2. Effects of acute (single dose) intragastric exposure to $Pt(SO_4)_2$ on open-field behavior of the mouse. $Pt(SO_4)_2$ was administered to males at the LD_5 (144 mg Pt/kg) or LD_{25} (213 mg Pt/kg) level. Ambulations and rearings were recorded over a 5 minute observation period at 4 hours or 1, 3, or 7 days post administration.

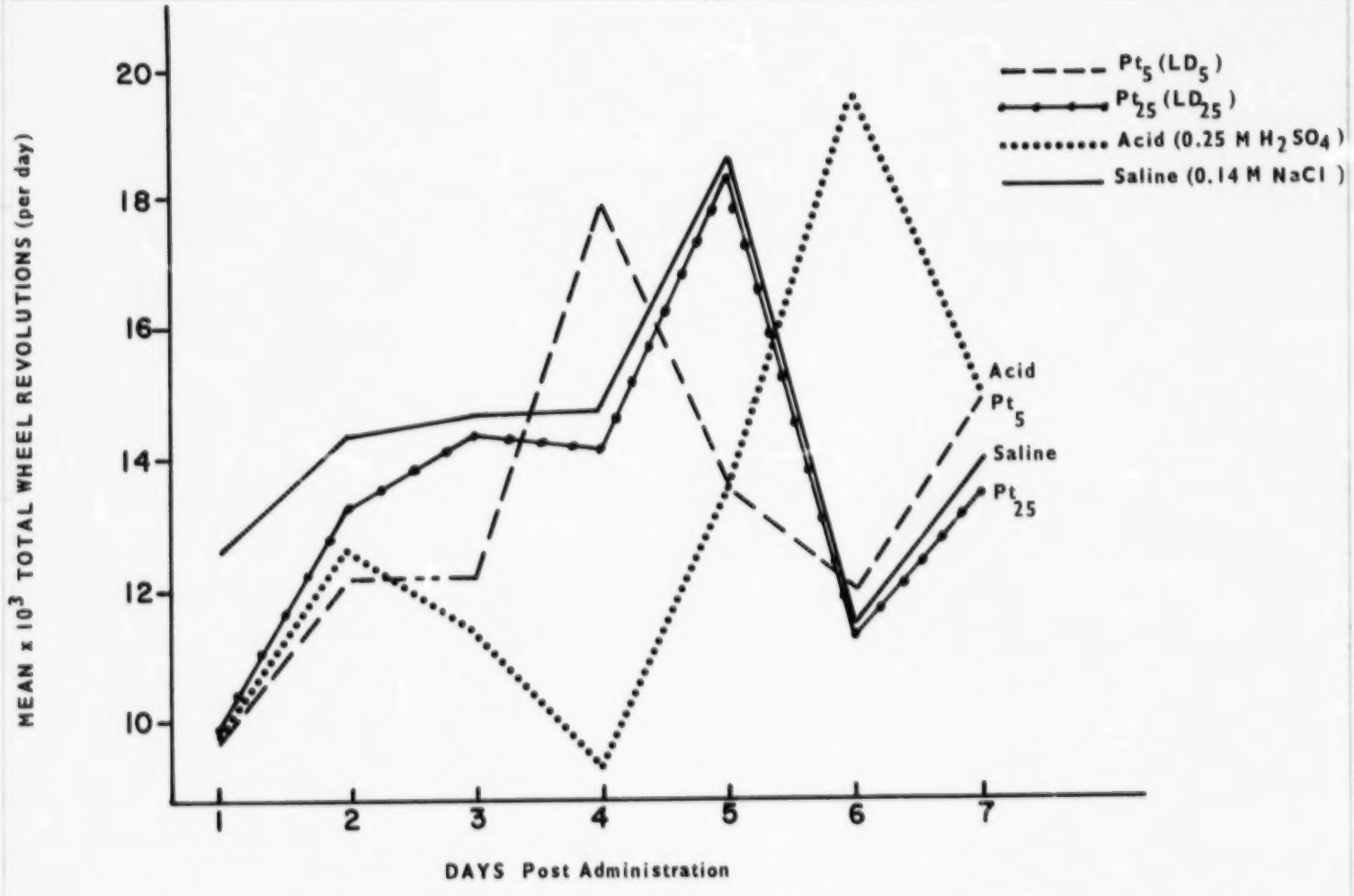


Figure 3. Wheel running performance for a 7 day period after administration of $Pt(SO_4)_2$, H_2SO_4 or NaCl.

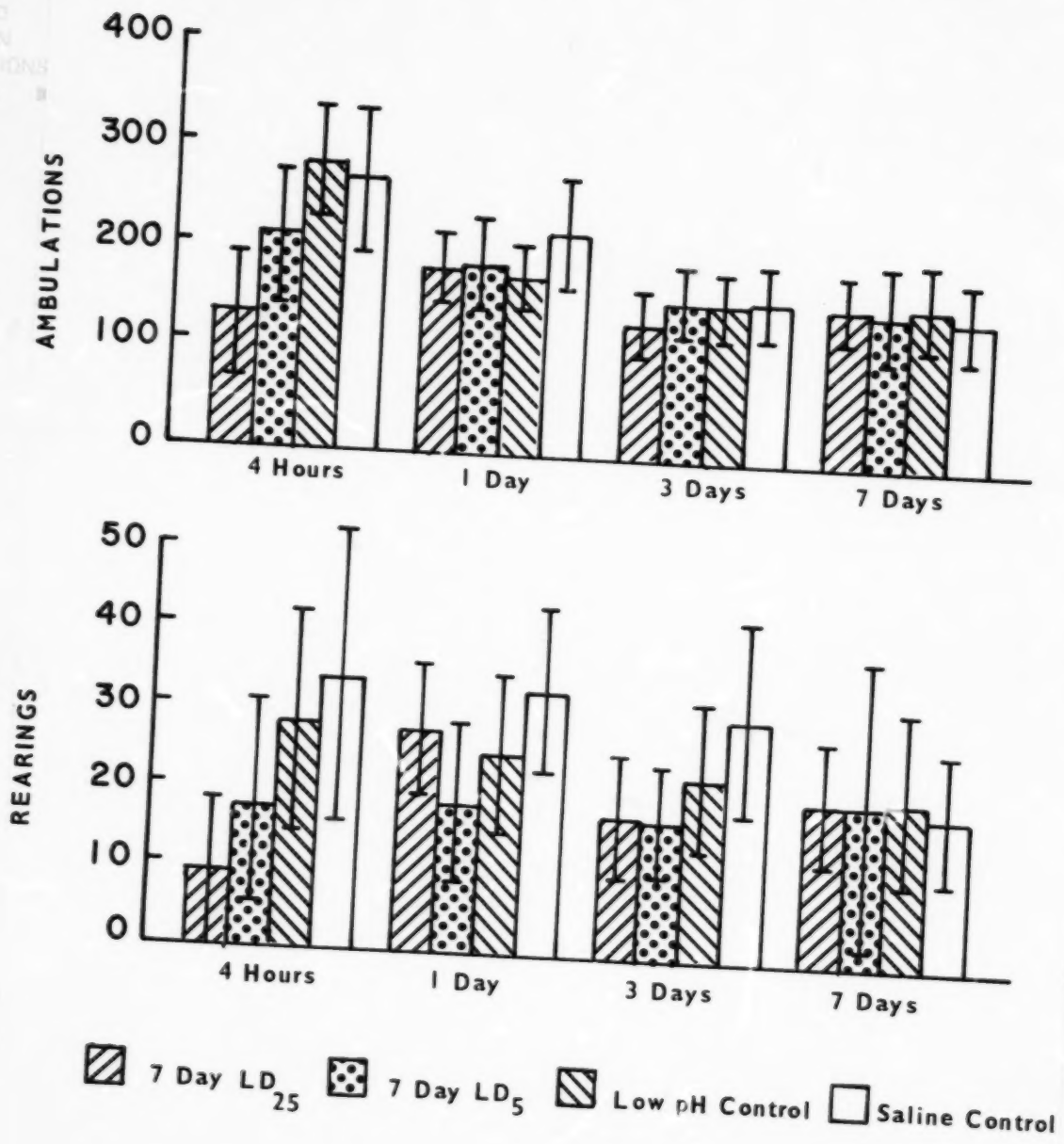


Figure 4. Effects of acute (single dose) intragastric exposure to Na_2PtCl_6 on open-field behavior of the mouse. Na_2PtCl_6 was administered to males at the LD_5 or LD_{25} level. Ambulations and rearings were recorded over a 5 minute observation period at 4 hours or 1, 3, or 7 days post administration.

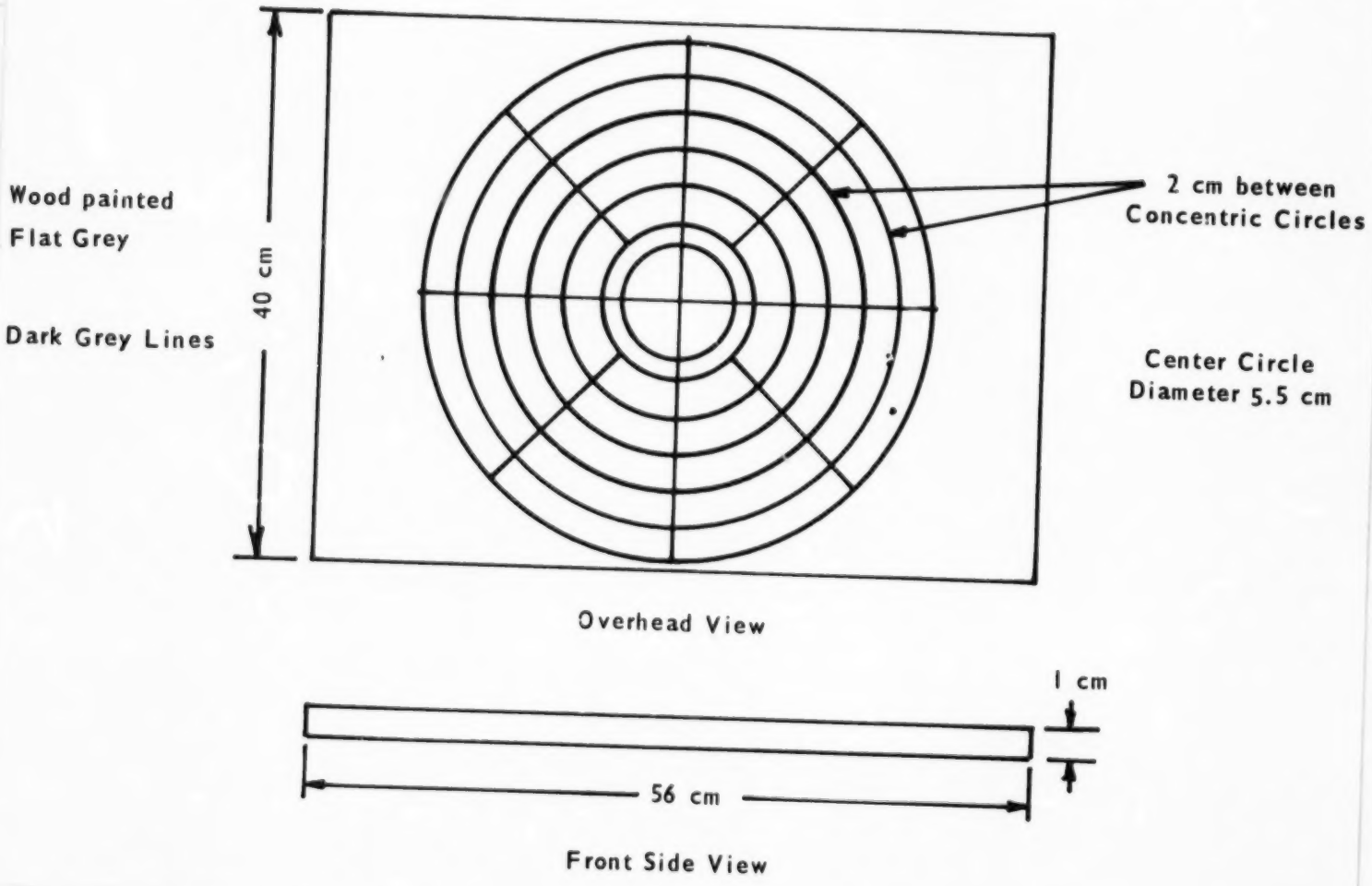
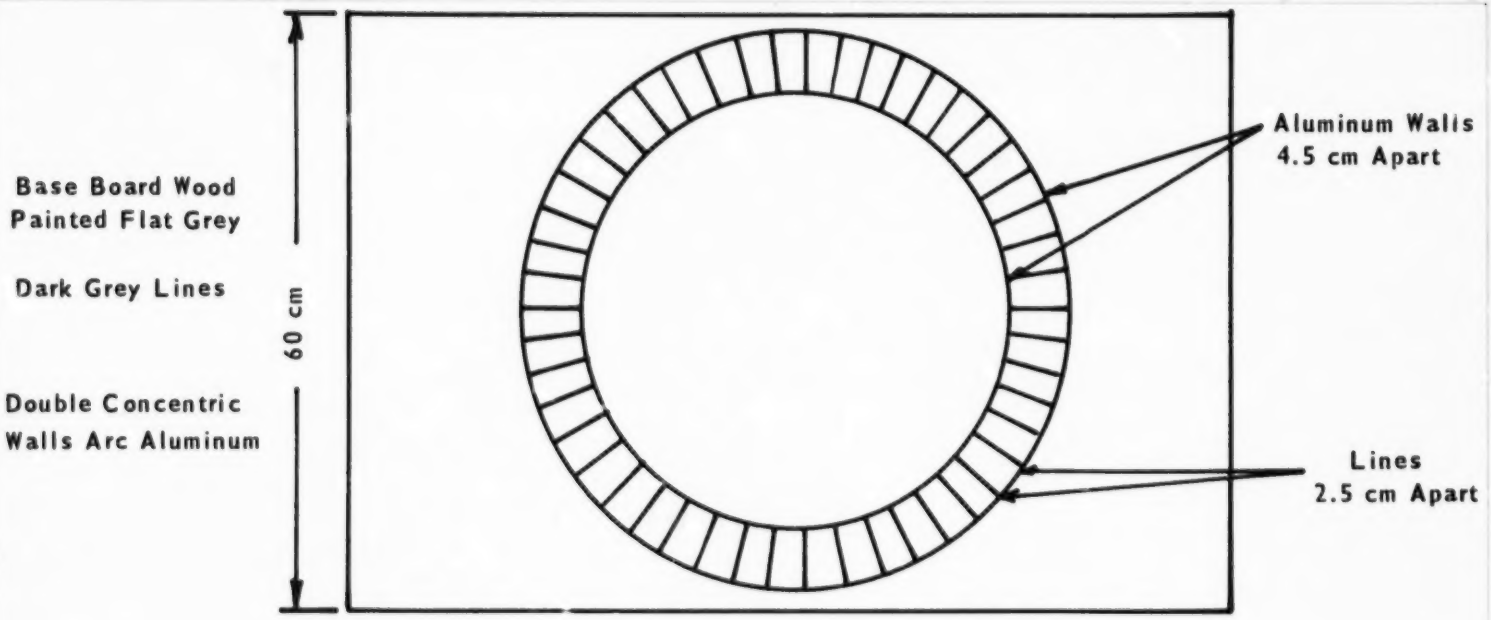


Figure 5. Activity board. The field was painted a light, flat grey. The circles and radii were dark grey. The center circle had a diameter of 5.5 cm.



Overhead View.

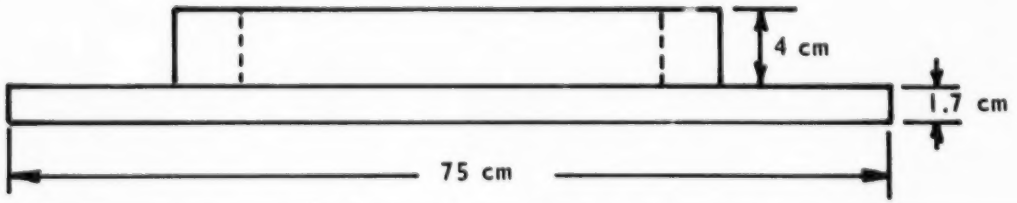


Figure 6. Circular runway. The runway was enclosed by concentric aluminum walls. the floor of the runway was painted a light, flat grey and the lines, dark grey.

TABLE 1. EFFECT OF INCREASING Pt (AS Na_2PtCl_6) DOSE[1] ON 7 DAY VIABILITY OF GRAVID[2] AND NONGRAVID ANIMALS

Dose Level (mg/kg)	Viability (Nongravid)	(Gravid)
15	10/10	10/10
20	10/10	9/9
25	8/10	6/7
30	7/10	6/9
35	1/10	5/11
40	0/9	1/7
45	0/10	0/7

[1] The Pt dose administered subcutaneously, was based on maternal weight at time of injection.

[2] All mice were injected on day 12 of gestation.

TABLE 2. LETHAL DOSE LEVELS FOR SINGLE DOSE EXPOSURE OF GRAVID AND NONGRAVID FEMALES TO Na_2PtCl_6: LINEAR REGRESSION ANALYSIS OF 7 DAY VIABILITY

Lethality[1]	Nongravid Dose[2,3]	Range[4]	Gravid Dose	Range
LD_1	21.4	12.3-25.0	21.8	8.9-25.4
LD_5	23.6	15.6-26.8	23.7	12.7-27.7
LD_{10}	24.9	17.7-27.8	25.4	15.3-29.1
LD_{25}	27.2	21.6-29.9	28.5	26.7-32.0
LD_{40}	28.9	24.6-31.9	30.9	25.0-34.8
LD_{50}	30.0	26.3-33.4	32.4	27.5-37.2
LD_{60}	31.1	27.9-35.3	34.0	29.8-40.4
LD_{75}	33.1	30.0-39.6	36.8	32.8-48.1
LD_{90}	36.1	32.7-48.0	41.3	36.3-64.4
LD_{95}	38.1	34.0-54.3	44.3	38.2-77.4
LD_{99}	42.1	36.5-68.8	50.4	41.7-110.0

[1] Lethality levels are expressed as the percent mortality expected within 7 days after Pt administration.

[2] Pt dose (in ppm) administered. Calculated to produce the corresponding lethality on the basis of maternal weight.

[3] All mice were injected on day 12 of gestation.

[4] The $p < 0.05$ probability range of the corresponding dose.

TABLE 3. ANALYSIS OF VARIANCE OF TISSUE/ORGAN Pt CONCENTRATIONS OF MALE ADULT MICE RECEIVING A SINGLE DOSE[1] OF $Pt(SO_4)_2$

Variable	Effects being tested		
	Time (Post administration)	Dose	Time by dose
Multivariate d.f. F-ratio	30 and 182.66 8.80^2	10 and 62 9.19^2	30 and 182.66 3.33^2
Univariate d.f. F-ratios	3 and 71	1 and 72	3 and 71
Blood	3.80^3	20.87^2	1.85
Liver	2.71^4	32.91^2	1.03
Spleen	1.12	43.29^2	0.79
Kidney	7.05^2	13.24^2	2.58
Lung	7.69^2	20.31^2	2.56
Testes	6.01^2	77.75^2	2.18
Muscle	8.04^2	28.73^2	5.09^3
Cerebrum	12.71^2	1.06	0.60
Cerebellum	45.61^2	1.96	5.75^3
Brain stem	12.03^2	1.48	0.31

[1] Either the 7 day i.g. LD_5 or LD_{25}

[2] $p < 0.001$

[3] $p < 0.01$

[4] $p < 0.05$

TABLE 4. TISSUE/ORGAN Pt CONCENTRATIONS (μgPt/g WET WEIGHT OF TISSUE) OF ADULT MALE MICE RECEIVING A SINGLE DOSE OF $Pt(SO_4)_2$

Dose	213 mg Pt/kg BW							
Time[2]	4 Hour		1 Day		3 Days		7 Days	
Tissue	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Blood	29.8	(31.6)	15.9	(14.0)	14.4	(12.8)	6.5	(3.5)
Liver	28.5	(22.7)	34.6	(33.0)	37.6	(26.6)	13.9	(7.9)
Spleen	12.7	(11.3)	12.8	(11.2)	18.7	(13.9)	10.8	(5.9)
Kidney	48.5	(51.7)	25.0	(17.7)	20.2	(12.5)	1.5	(0.7)
Lung	27.2	(18.5)	16.4	(20.2)	8.9	(6.8)	3.8	(1.8)
Testes	4.3	(2.6)	2.8	(2.1)	3.1	(1.2)	5.8	(2.2)
Muscle	3.7	(2.5)	2.1	(2.4)	1.0	(0.7)	0.4	(0.2)
Cerebrum	1.4	(1.4)	0.2	(0.4)	0.1	(0.2)	0.2	(0.1)
Cerebellum	1.6	(0.8)	0.0	(0.0)	0.1	(0.2)	0.3	(0.2)
Medulla	0.8	(0.7)	0.2	(0.4)	0.0	(0.1)	0.2	(0.1)

Dose	144 mg Pt/kg BW							
Time[2]	4 Hour		1 Day		3 Days		7 Days	
Tissue	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Blood	5.9	(4.3)	3.4	(4.2)	2.1	(0.9)	1.2	(1.0)
Liver	8.1	(5.1)	8.6	(12.1)	4.9	(2.6)	1.0	(1.3)
Spleen	2.2	(1.3)	2.7	(3.2)	2.4	(1.1)	1.3	(0.5)
Kidney	12.8	(6.2)	7.5	(7.2)	6.3	(7.4)	1.3	(1.3)
Lung	7.4	(8.3)	2.6	(2.8)	1.8	(0.5)	1.4	(2.0)
Testes	1.3	(1.2)	0.7	(0.7)	0.5	(0.2)	1.3	(1.0)
Muscle	0.6	(0.6)	0.3	(0.3)	0.2	(0.1)	0.1	(0.1)
Cerebrum	0.9	(1.2)	0.1	(0.2)	0.0	(0.0)	0.2	(0.1)
Cerebellum	0.9	(0.5)	0.3	(0.3)	0.0	(0.1)	0.2	(0.2)
Medulla	0.6	(0.8)	0.1	(0.1)	0.1	(0.1)	0.1	(0.1)

[1] Either the 7 day i.g. LD_5 or LD_{25}

[2] Post administration

TABLE 5. TISSUE/ORGAN Pt CONCENTRATIONS (μgPt/g WET WEIGHT OF TISSUE) OF ADULT MALE MICE RECEIVING 1-10 DOSES[1] OF $Pt(SO_4)_2$

Number of doses	Tissue/Organ													
	Blood		Liver		Kidney		Spleen		Lung		G.I. Tract		Testes	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
2	0.19	0.11	0.15	0.13	1.88	2.12	0.23	0.29	1.23	3.27	0.13	0.09	0.10	0.12
4	0.50	0.32	0.35	0.26	2.71	1.67	0.72	0.43	1.40	2.65	0.16	0.12	0.28	0.17
6	1.18	0.63	0.58	0.32	3.90	1.78	1.95	1.69	3.30	7.78	1.04	1.78	0.53	0.34
8	1.07	1.05	1.22	1.33	6.84	4.86	2.61	3.69	4.51	5.56	0.29	0.27	0.60	0.35
10	1.03	0.77	0.96	0.53	8.29	8.48	2.11	1.70	8.30	8.91	0.45	0.50	0.57	0.36

[1] $Pt(SO_4)_2$ was administered i.g. at the LD_1 level

TABLE 6. ANALYSIS OF VARIANCE OF TISSUE/ORGAN Pt CONCENTRATIONS OF ADULT MALE MICE RECEIVING A SINGLE DOSE OF Na_2PtCl_4

Variable	Effects being tested		
	Time	Treatment	Time x Treatment
Multivariate d.f.	12/177.56	4/67	12/177.56
F-ratio	5.72	2.95[3]	.92
Univariate d.f.	3/70	1/70	3/70
F-ratios			
Blood	3.62[4]	2.36	——
Liver	7.77[2]	3.07	——
Spleen	0.45	7.40[3]	——
Kidney	6.90[2]	8.33[3]	——

[1] Either the 7 day i.g. LD_5 or LD_{25}

[2] $p < .001$

[3] $p < .01$

[4] $p < .05$

TABLE 7. TISSUE LEVELS OF Pt FOLLOWING MATERNAL ADMINISTRATION OF A SINGLE DOSE[1] OF Na_2PtCl_6 ON DAY 7 OF GESTATION

	Days post administration			
	1	2	3	5
	Day of gestation			
	8	9	10	12
Blood[2]	8.70±1.07	4.40±1.14	4.26±1.80	0.94±0.06
Liver[2]	14.60±0.60	15.80±3.50	17.20±5.30	8.82±0.92
Kidney[2]	63.10±2.80	38.30±5.90	41.30±8.90	34.70±2.30
Spleen[2]	6.43±0.72	7.80±0.96	11.70±4.40	6.65±3.25
Brain[2]	-[4]	-	-	-
Embryo[3]	5.56±0.43	3.56±0.44	3.40±0.92	-[5,6]
Fetus[3]	-	-	-	-[4]
Placenta[3]	-	-	-	2.50±0.90[6]

[1] The 7 day s.c. LD_1.

[2] All values are in parts per million Pt based on tissue wet weight. Each value is the mean ± the standard error of the mean for 5 animals.

[3] All values are in ppm wet weight. Each value is the mean ± SEM for all embryos/fetuses/placentas from 5 mothers.

[4] Undetectable. Limit of detection less than 0.1 ppm.

[5] Not assayed.

[6] The day 12 conceptus may be separated into fetal and placental components.

TABLE 8. TISSUE LEVELS OF Pt FOLLOWING MATERNAL ADMINISTRATION OF A SINGLE DOSE[1] OF Na_2PtCl_6 ON DAY 12 OF GESTATION

	Days post administration			
	1	2	3	5
	Day of gestation			
	11	12	13	15
Blood[2]	5.10±0.89[1]	2.92±0.45	3.15±0.56	0.89±0.09
Liver[2]	22.40±3.20	20.10±0.60	18.40±4.20	8.16±0.85
Kidney[2]	49.40±1.30	41.30±4.20	41.80±2.70	30.40±2.90
Spleen[2]	9.03±0.79	11.30±2.90	12.50±2.40	13.80±3.10
Brain[2]	0.43±0.01	–[4]	–[4]	–[4]
Fetus	0.22±0.07	–[4]	–[4]	–[4]
Placenta	6.90±1.20	4.31±1.00	4.95±0.75	3.73±0.71

[1] The 7 day s.c. LD_1.

[2] All values are in parts per million based on tissue wet weight. Each value is the mean ± the standard error of the mean for 5 animals.

[3] All values are in ppm wet weight. Each value is the mean ± SEM for all embryos/fetuses/placentas from 5 mothers.

[4] Undetectable. Limit of detection less than 0.1 ppm.

TABLE 9. WHOLE BODY Pt LEVELS IN NEONATES FROM DAMS RECEIVING A SINGLE DOSE[1] OF Na_2PtCl_6 ON DAY 2 POST PARTUM

	Days after administration		
	1	3	5
	Days post partum		
	3	5	7
Male[2]	0.37±0.08	0.31±0.13	0.10±0.06
Females[2]	0.24±0.06	0.29±0.13	0.10±0.08

[1] The 7 day s.c. LD_1.

[2] All values are in parts per million based on tissue wet weight. Each value is the mean ± the standard error of the mean for 5 animals.

TABLE 10. EFFECTS OF A SINGLE DOSE[1] OF $Pt(SO_4)_2$ ON OPEN FIELD BEHAVIOR: ANALYSIS OF VARIANCE

Variable	Effects being tested		
	Time	Treatment	Time by treatment
Multivariate d.f.	6 and 286	6 and 286	18 and 286
F-ratio	2.76 ($p < 0.01$)	2.61 ($p < 0.02$)	0.69 (N.S.)
Univariate d.f.	3 and 144	3 and 144	9 and 144
F-ratios			
Ambulations	3.68 ($p < 0.01$)	4.85 ($p < 0.005$)	——
Rearings	2.38 ($p < 0.07$)	2.43 ($p < 0.07$)	——

[1] $Pt(SO_4)_2$ was administered i.g. at the LD_5 or LD_{25} level.

TABLE 11. CORRELATIONS BETWEEN OPEN FIELD BEHAVIORAL MEASURES AND TISSUE/ORGAN Pt CONCENTRATIONS IN ANIMALS RECEIVING A SINGLE DOSE[1] OF $Pt(SO_4)_2$

Tests of the hypothesis of no association between behavior and tissue Pt concentrations	Overall: F = 2.45, p < 0.001, d.f. = 20 and 134	
	Ambulations: F = 3.98, p < 0.001, d.f. = 10/68	Rearings: F = 3.35, p < 0.001, d.f. = 10/68
Correlation coefficients	d.f. = 78	
Blood	-0.487[2]	-0.394[2]
Liver	-0.452[2]	-0.346[2]
Spleen	-0.553[2]	-0.469[2]
Kidney	-0.384[2]	-0.271[3]
Lung	-0.413[2]	-0.345[2]
Testes	-0.476[2]	-0.390[2]
Muscle	-0.323[2]	-0.216
Cerebrum	-0.159	-0.097
Cerebellum	-0.211	-0.125
Brain stem	-0.195	-0.163

[1] $Pt(SO_4)_2$ was administered i.g. at the LD_5 or LD_{25} level

[2] $p < 0.01$

[3] $p < 0.05$

TABLE 12. EFFECTS OF A SINGLE DOSE[1] OF Na_2PtCl_6 ON OPEN FIELD BEHAVIOR: ANALYSIS OF VARIANCE

Variables	Effects being tested		
	Time	Treatment	Time x treatment
Multivariate d.f.	6/282	6/282	18/282
F-ratio	14.01[2]	6.35[2]	2.99[2]
Univariate d.f.	3/142	3/142	9/142
F-ratios			
Ambulations	19.78[2]	8.65[2]	5.02[2]
Rearings	1.97	7.69[2]	2.39[3]

[1] Na_2PtCl_6 was administered i.g. at the LD_5 or LD_{25} level.

[2] $p < 0.001$

[3] $p < 0.05$

TABLE 13. EFFECTS OF EXPOSURE TO MULTIPLE (SERIALLY REPEATED) DOSES[1] OF $Pt(SO_4)_2$ ON OPEN FIELD (AMBULATIONS AND REARINGS) AND EXPLORATORY BEHAVIORS OF THE MOUSE

	Ambulations					
	Platinum		Low pH		Saline	
Doses	Mean	SD	Mean	SD	Mean	SD
1	172.0	42.8	174.4	18.6	160.0	27.3
2	193.6	49.6	190.8	28.7	174.6	29.8
3	181.2	34.6	195.3	49.9	208.2	46.1
4	190.0	43.6	190.2	35.3	175.6	38.7
5	171.5	34.7	180.6	44.9	184.0	78.2
6	196.1	32.7	189.3	59.6	207.2	76.8
7	134.9	57.0	196.6	58.7	186.2	49.5
8	165.9	24.9	175.3	39.7	187.2	34.6
9	141.4	47.2	176.6	34.1	184.0	43.2
10	176.7	30.2	199.2	53.3	200.8	36.4

	Rearings					
	Platinum		Low pH		Saline	
Doses	Mean	SD	Mean	SD	Mean	SD
1	40.1	16.6	38.2	19.9	29.4	7.9
2	42.3	9.4	51.5	15.2	36.8	16.6
3	40.7	11.6	37.1	8.9	35.8	6.8
4	42.9	13.6	35.6	12.7	31.6	14.8
5	37.8	9.1	33.8	8.0	39.2	19.9
6	33.6	13.1	35.6	18.1	31.6	13.7
7	25.2	15.6	33.2	12.6	45.0	11.4
8	25.6	8.9	23.4	10.0	34.2	14.8
9	21.6	8.3	30.3	9.3	27.8	13.7
10	23.3	6.8	33.7	13.9	39.6	7.4

(continued)

TABLE 13. (continued)

Doses	Explorations: Platinum Mean	Platinum SD	Low pH Mean	Low pH SD	Saline Mean	Saline SD
1	29.4	10.9	30.9	10.8	36.6	15.5
2	28.7	17.6	40.6	12.2	42.2	15.4
3	37.6	16.7	29.5	18.7	33.4	14.4
4	38.2	19.5	28.1	14.1	20.0	7.9
5	27.2	15.6	31.8	20.2	36.2	19.2
6	27.1	14.6	41.0	13.3	43.4	26.8
7	28.8	20.1	38.2	10.5	40.0	16.9
8	35.5	20.9	35.6	13.1	34.6	12.0
9	30.1	22.8	33.6	17.1	34.8	16.4
10	17.9	15.6	42.4	14.1	47.4	11.9

[1] $Pt(SO_4)_2$ was administered i.g. at the LD_1 level

TABLE 14. EFFECTS OF EXPOSURE TO MULTIPLE (SERIALLY REPEATED) DOSES[1] OF $Pt(SO_4)_2$ ON BEHAVIORAL MEASURES: ANALYSIS OF VARIANCE[2]

Variable	Effects being tested		
	Number of doses	Treatment	Doses x treatment
Multivariate d.f.	27/637.31	6/436	54/650.37
F-ratio	2.37 ($p<0.001$)	1.97 (N.S., $p<0.07$)	1.27 (N.S., $p<0.10$)
Univariate d.f.	9/220	2/220	18/220
F-ratios[3]			
Ambulations	1.61 (N.S.)	3.29 ($p<0.05$)	0.83 (N.S.)
Rearings	5.04 ($p<0.001$)	0.66 (N.S.)	1.69 ($p<0.05$)
Explorations	0.42 (N.S.)	3.86 ($p<0.05$)	1.49 (N.S., $p<0.10$)

[1] $Pt(SO_4)_2$ was administered i.g. at the LD_1 level.

[2] The data were analyzed by a two-way analysis of variance. The factors were: number of dose and treatment [$Pt(SO_4)_2$, low pH, saline].

[3] Univariate F-ratios normally are not examined if the corresponding multivariate F-ratio is not significant at the $p<0.05$ level. However, in these studies, while formally retaining the critical level of $p<0.05$, we are noting significance levels up to $p<0.10$.

TABLE 15. CORRELATIONS BETWEEN OPEN FIELD AND EXPLORATORY BEHAVIORAL MEASURES AND TISSUE Pt LEVELS: $Pt(SO_4)_2$ MULTIPLE (SERIALLY REPEATED) DOSE STUDY

Tests of no association between behavioral & tissue measures	Overall F = 1.683, d.f. = 21/115.4085, $p < .05$		
	Ambulations F=1.24, df=7/42 N.S.	Rearings F=2.80, df=7/42 $p < .02$	Explorations F=1.47, df=7.42 N.S.
Correlation coefficients (df = 49)			
Blood	-0.132	-0.285[2]	-0.088
Liver	-0.210	-0.425[1]	0.112
Kidney	-0.259	-0.371[1]	-0.237
Spleen	-0.194	-0.301[2]	-0.035
Lung	-0.231	-0.232	-0.357[1]
GI	0.010	-0.025	-0.044
Testes	-0.228	-0.378[1]	-0.047

[1] = $p < 0.01$

[2] = $p < 0.05$

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